



Original/Investigación animal

Histopathology of gastric wall in Chinese alligator *Alligator sinensis* infected with *Ortleppascaris sinensis* (Nematoda: Ascaridoidea)

Jinhong Zhao^{1,2}, Shaosheng Wang¹, Genjun Tu³, Yongkang Zhou³, Xiaobing Wu^{2*} and Chaopin Li¹

¹Department of Parasitology, Wannan Medical College, Wuhu, Anhui. ²Provincial Laboratory of Conservation and Exploitation of Biological Resources, College of Life Sciences, Anhui Normal University, Wuhu, Anhui. ³The National Nature Reserve of Chinese Alligator in Anhui, Xuanzhou, Anhui (China).

Abstract

Crocodiles are susceptible to infection with a wide array of external and internal gastrointestinal helminths, yet little is known on the histopathology following infection or the effects of these parasites. The present study was aimed at evaluating the impact of infection by *Ortleppascaris sinensis* (Nematoda: Ascaridoidea) on the stomach of captive *Alligator sinensis*. The histological examination of the stomach revealed presence of superficial ulcer in mucous layer and granulomatous inflammation in submucous layer at entire gastric walls of the *Alligator sinensis*. Our findings also confirm that development of *Ortleppascaris sinensis* is in close association with the wall of the stomach.

(Nutr Hosp. 2015;32:1180-1183)

DOI:10.3305/nh.2015.32.3.9344

Key words: Histopathology. *Ortleppascaris sinensis*. Stomach.

Introduction

Crocodiles are susceptible to infection with a wide variety of external and internal protozoan and metazoan parasites¹. Although many parasites are considered to be of low or nil pathogenicity, or sub clinical, and not the cause of significant disease², some may affect a wide range of organs and tissues, consequently resulting in traumatic injury or vulnerability to secondary infections, for which many parasites can be responsible for illness and death. For example, infections caused by Protozoa, such as the genera *Trypanosoma* and *Leishmania*, within the order Kinetoplastida, may even lead to ill and death thrift in hatchlings in farmed *Crocodylus porosus* in Northern Australia and Papua New

Correspondence/Correspondencia: Xiaobing Wu.
College of Life Sciences, Anhui Provincial Laboratory of Conservation and Exploitation of Biological Resources.
Anhui Normal University, Wuhu.
Anhui 241000, China.
E-mail: wuxb@mail.ahnu.edu.cn

Recibido: 3-VI-2015.
Aceptado: 26-VI-2015.

HISTOPATOLOGÍA DE LA PARED GÁSTRICA EN EL ALIGÁTOR CHINO ALLIGATOR SINENSIS INFECTADO CON ORTLEPPASCARIS SINENSIS (NEMATODOS: ASCARIDOIDEA)

Resumen

Los cocodrilos son susceptibles a la infección con una amplia gama de helmintos gastrointestinales externos e internos; sin embargo, poco se sabe sobre la histopatología tras la infección o los efectos de estos parásitos. El presente estudio pretende evaluar el impacto de la infección por *Ortleppascaris sinensis* (Nematodos: Ascaridoidea) sobre el estómago del *Alligator sinensis* en cautividad. El examen histológico del estómago reveló la presencia de úlcera superficial en la capa mucosa e inflamación granulomatosa en la capa submucosa del conjunto de las paredes gástricas del *Alligator sinensis*. Nuestros resultados confirman también que el desarrollo de *Ortleppascaris sinensis* está estrechamente relacionado con la pared del estómago.

(Nutr Hosp. 2015;32:1180-1183)

DOI:10.3305/nh.2015.32.3.9344

Palabras clave: Histopatología. *Ortleppascaris sinensis*. Estómago.

Guinea³. Crocodylians were reported to be infected by a number of different trematodes such as *Acanthostomum loossi* that were recovered from farmed *Crocodylus acutus* and *C. rhombifer* in Cuba, this trematode may cause poor health and low growth rates⁴. Gastrointestinal nematode infections are usually asymptomatic in crocodiles but may be occasionally associated with disease. For example, infection with *Dujardinascaris* may be associated with gastric ulceration and runting in hatchlings^{5,6}. There are loss of appetite and mortality of the Chinese alligator because of nematodes in its alimentary tract⁷. Considerable literatures on parasites of crocodylians can be available, yet they mainly focus on taxonomic interest, parasitic fauna and associated diseases, and little attention has ever been paid on the host response to the parasite or pathological effects due to infections.

The Chinese alligator, *Alligator sinensis* Fauvel, 1879 (Crocodilian: Alligatoridae) is exclusively native to eastern China and primarily distributed in the middle and lower reaches of the Yangtze River and Taihu Lake. Little is known about the parasite fauna of Chinese alligators^{1,8}. We first described a new nematode species, *Ortleppascaris sinensis* (Ascaridoidea) found in the stomach and intestine of the Chinese alligator *Alligator sinensis* in the National Nature Reserve of Chinese Alligator (Chinese Crocodile Lake) in Anhui Province, China⁹. The present study was undertaken to understand the pathopoiesis of the intestinal parasites *Ortleppascaris sinensis* through investigating the histopathological alterations of the gastric wall of *Alligator sinensis* infected with such species.

Materials and Methods

Collection of the parasites

Seven Chinese alligators *Alligator sinensis* Fauvel, 1879 (Crocodilian: Alligatoridae) were collected from the National Nature Reserve of Chinese Alligator in Anhui, Xuanzhou, China, between September 2011 and August 2014. The Chinese alligators were immediately anesthetized with pentobarbital and subsequently killed for examination of the pathopoiesis of intestinal parasites. The nematodes were recovered from digestive tracts, washed in PBS, and then fixed in hot 70% ethanol. The present study was approved by the National Nature Reserve of Chinese Alligator of Anhui Province.

Histological preparation

Gastric samples of *Alligator sinensis* with either parasitism or non-parasitism were collected and rinsed in phosphate-buffered saline buffer (PBS), and then fixed in 10% formalin overnight, followed by dehydration paraffin embedment. The paraffins were sectioned into 5 μ m thicknesses using a microtome. These sections were stained with hematoxylin and eosin (H&E), examined with an Olympus BX 51 microscope (Olympus, Shibuya-Ku, Tokyo, Japan), and photographed using a digital camera. The inflammatory changes were microscopically examined and assessed based on the extent of inflammatory cell infiltration and tissue damage in the stomach.

Results

Identification of the parasites

Although there were no definitive clinical manifestations indicative of any infections in these seven Chinese alligators, we did find that four were infected

with some nematodes, which were identified as the same species, namely *Ortleppascaris sinensis*⁹ by their morphology and molecular characterization.

Histological findings in the parasitized stomach

Stomachs were taken from the Chinese alligators. Gross appearance of the gastric wall infected with *Ortleppascaris sinensis* indicated smooth gastric mucosa and regular plica. However, some of the gastric walls were found with multifocal ulceration to a certain degree. Further examination of samples from normal and diseased gastric tissues were executed under the microscope. We found that most structure of the mucosa, submucosa, muscular layer and tunica adventitia remained intact in the gastric tissues free of nematode infection (Fig. 1a and b). The epithelium in the gastric mucosa surface consisted of simple columnar cells, and submucosa was covered with loose connective tissues, biggish vas and nerve. The muscular gastric wall appeared thicker and comprised oblique muscle, circular muscle and longitudinal muscle from internal to external. However, there were multifocal superficial erosions (Fig. 1c), mucosa being covered with funnel-like depression in the lesion (Fig. 1d) or some granulomatous inflammation (Fig. 1e and f) in the gastric wall from Chinese alligators infected with nematodes. The diameter of the lesion ranged from 0.52 mm to 0.95 mm. The inflammatory exudations and sphacelus were filled in superficial erosions. However, the structure of mucosa depression remained intact, though it being thin and sunken. This change was the result of regeneration of the repaired gastric mucosa (Fig. 1d). The granulomatous inflammation was seen in the mucosa depression region, and located in the submucosa (Fig. 1d). There was a central area of necrosis surrounded by a large number of epithelioid cells, multinuclear cells and massive inflammatory cells infiltration. The inflammatory cells were predominated by lymphocyte, monocyte and bits of eosinophils. Fibrillar connective tissues were seen hyperplastic, with peripherally spread collagen and expanded and congestive blood vessels (Fig. 1e and f).

Discussion

Granulomatous inflammation is a chronic hyperplastic condition, primarily characterized by granuloma formation. This inflammation may arise as a result of the infection with bacteria, spirochete, fungus, parasites, foreign matter and other unknown cause¹⁰. Our researches revealed superficial ulcer in the mucous layer and granulomatous inflammation in submucous layer of all gastric walls of Chinese alligator affected by *Ortleppascaris sinensis*. There was a central area of eosinophilic necrosis surrounded by a large number of inflammatory cell infiltration. These features were especially well defined when compared with the

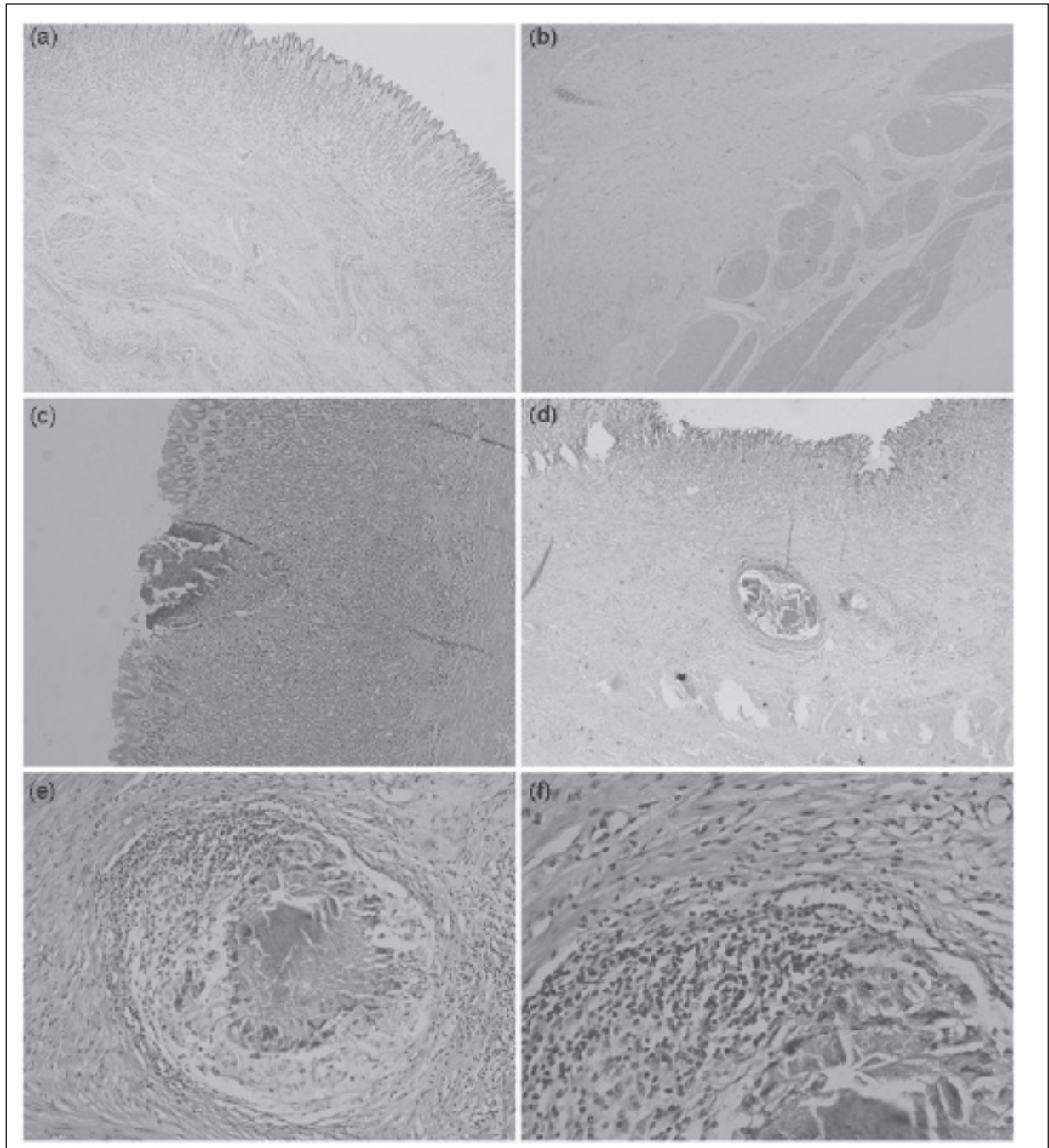


Fig. 1.—Observation of gastric wall tissue sections (HE staining). (a) Normal (mucosa and submucosa layer, $\times 40$); (b) Normal (submucosa and muscular layer, $\times 40$); (c) Superficial erosions (mucosa layer, $\times 100$); (d) Granulomatous inflammation (mucosa and submucosa layer, $\times 40$); (e) Granulomatous inflammation (submucosa layer, $\times 200$); (f) Granulomatous inflammation (submucosa layer, $\times 400$).

non-parasitized gastrointestinal tract. The structure of the non-parasitized gastric wall of *Alligator sinensis* was normal, histologically similar to the configuration in gastrointestinal tract of *Alligatoe sinensis* described by Chen ¹¹.

Species of adult *Ortleppascaris* have been found in the gastrointestinal tract of alligators and crocodiles ^{12,13,1,9}, and larval forms were found in the liver of

amphibians ^{14,15} as well as the mesenteries and the body cavity of naturally infected fishes in Africa and frogs in the USA ^{16,17}. While the life cycle of this parasite is still not completely clear. Sprent (1977) suggested that it would seem likely that the life cycle of *Dujardinascaris* involves an encysted stage in the tissues of fish, frogs, or other food animals, when swallowed by crocodiles, the third stage emerges. The fourth stage and

early adult stage probably occurs in close association with the wall of the stomach. *Ortleppascaris* is removed by Sprent (1978) from the genus *Dujardinascaris*¹⁸ and placed in a new genus *Ortleppascaris* Sprent, 1978. So there is a great similarity about the life cycle between the genus *Ortleppascaris* and *Dujardinascaris*. Our study that superficial ulcer in mucous layer and granulomatous inflammation in submucous layer as proof of this. It is probable that fishes, frogs and other food animals serve as intermediate hosts for *Ortleppascaris*, and the fourth stage develops for a while in submucous layer of gastric wall, and then returns to the lumen to develop to adult. The study shows that nematode *Ortleppascaris sinensis* can cause granulomatous inflammation of gastric wall in Chinese alligator; also provide some foundation for further research the life cycle of *Ortleppascaris sinensis*.

Financial support

This study was supported by the National Natural Science Foundation of China (No: 31272337 and 31472019) and Key Project of Wannan Medical University of China (No: WK2013Z04).

Conflict of interest

None.

References

1. M., T., *A Checklist of Host-Parasite Interactions of the Order Crocodylia*. 2013, California: University of California Press. 273.
2. Cubas, Z.S., Special challenges of maintaining wild animals in captivity in South America. *Rev Sci Tech*, 1996. 15(1): 267-87.
3. Ladds, P.W., J.A. Donovan, A. Reynolds and J.A. Turton, Giant cell enteritis in young crocodiles. *Aust Vet J*, 1994. 71(9): 300-1.
4. I., P.-B., S. G., B. A. and B. I.P., Report and course of an acute parasitic infection due to *Acanthostomum loossi* in young animals from a commercial crocodile hatchery (*C. acutus* and *C. rhombifer*). *Revista Cubana de Ciencias Veterinarias*, 1980. 11: 79-83.
5. Ladds, P.W. and L.D. Sims, Diseases of young captive crocodiles in Papua New Guinea. *Aust Vet J*, 1990. 67(9): 323-30.
6. Huchzermeyer, F.W., *Crocodiles: Biology, Husbandry, and Diseases*. 2003, London: CABI publishing.
7. R.P., W., J.S. Nie and C.L. Wang, Ascariasis of Chinese alligators (In Chinese). *Herpetology Sinica*, 1995. 4: 350-1.
8. Zhao, J., Y. Zhou, S. Wang, G. Tu, X. Tang and X. Wu, Preliminary report on the intestinal parasites and their diversity in captive Chinese alligators. *Nutr Hosp*, 2014. 31(2): 813-9.
9. Zhao, J.H., S.S. Wang, G.J. Tu, Y.K. Zhou and X.B. Wu, Morphological and molecular characterization of *Ortleppascaris sinensis* sp. nov. (Nematoda: Ascaridoidea) from the Chinese alligator *Alligator sinensis*. *J Helminthol*, 2015: 1-9.
10. El-Dakhly Kh, M., E. El-Nahass, S. Uni, H. Tuji, H. Sakai and T. Yanai, Levels of infection of gastric nematodes in a flock of great cormorants (*Phalacrocorax carbo*) from Lake Biwa, Japan. *J Helminthol*, 2012. 86(1): 54-63.
11. B.H., C., H. T.M., W. X.B. and W. C.L., *Research on the Chinese alligator (In Chinese)*. 2003, Shanghai: Technology Education Press.
12. Sprent, J.F., Ascaridoid nematodes of amphibians and reptiles: *Gedoelestascaris* n.g. and *Ortleppascaris* n.g. *J Helminthol*, 1978. 52(3): 261-82.
13. Waddle, A.R., J.M. Kinsella, J.P. Ross, E. Rojas-Flores, H.F. Percival and D.J. Forrester, Nematodes collected by gastric lavage from live American alligators, *Alligator mississippiensis*, in Florida. *J Parasitol*, 2009. 95(5): 1237-8.
14. Moravce, F. and H. Kaiser, Helminth parasites from West Indian frogs, with descriptions of two new species. *Caribbean Journal of Science*, 1995. 31: 252-68.
15. Silva, J.P., D.C. da Silva, F.T. Melo, E.G. Giese, A.P. Furtado and J.N. Santos, Liver histopathology in the cane toad, *Rhinella marina* (Amphibia: Bufonidae), induced by *Ortleppascaris* sp. larvae (Nematoda: Ascarididae). *J Parasitol*, 2013. 99(2): 250-6.
16. H.A., B., Some parasitic worms, mainly from fishes, from Lake Tanganyika. *Annals and Magazine of Natural History*, 1928. 15: 552-6.
17. N.E.E., M., Crocodiles as a source of nematode larval infestation among fish species in Lake Naser, Egypt. *Assiut Journal of Veterinary Medicine*, 1999(40): 88-102.
18. Sprent, J.F.A., Ascaridoid nematodes of amphibians and reptiles: *Dujardinascaris*. *Journal of Helminthology*, 1977. 51: 253-87.