Survey on the parasites and evaluate the pathopoiesis of parasites to Chinese alligators

Jinhong Zhao

Provincial Laboratory of Conservation and Exploitation of Biological Resources,
College of Life Sciences, Anhui Normal University, Wuhu, Anhui 241000, China

Introduction

Animal parasitic disease has characteristics of easy infection and transmission diversity, and makes it to become the major disease to wildlife (Leclaire & Faulkner, 2014; Magnino *et al.*, 2009). Crocodiles, in common with other reptiles, usually harbor a wide variety of protozoa and metazoans external and internal parasites (Tellez, 2013). The Chinese alligator, *Alligator sinensis* Fauvel, 1879, a unique animal in China, is listed as a critically endangered species. Unfortunately, little attention has ever paid on the parasitic study, and such reports are rare, either. It has been reported in the literature the occurrence of parasite infection in crocodiles is available from Wang *et al*. (2007)(Wang Q, 2007)， who collected fecal samples of reptiles from Chengdu Zoo, Sichuan, China, including snakes, turtles and crocodiles etc., and incidentally identified the cysts on amoeba in one fecal sample of captive Chinese alligators.

Crocodilians were considered to have certain resistance or self-limited, and the damage of some parasites are low pathogenic, no pathogenicity, subclinical infection or does not cause disease to crocodilians. However, there are still some parasites can infect organ and tissue of the crocodilians, and causing trauma, secondary infection or even death (Cubas, 1996). Considerable literatures on parasites of crocodilians 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.

In order to understand the prevalence of the parasites and evaluate the pathopoiesis of parasites to Chinese alligators, we collected the fecal samples of captive Chinese alligators and dissected some carcas between 2017 and 2020 in a captive breeding center in south Anhui province, with an attempt to provide evidence for controlling or preventing the parasitic disease and conservation of this endangered species.

Methods

Study site

Chinese alligator, one of the known living species of alligators, is native only to eastern China and primarily distributed in the middle and lower reaches of the Yangtze River and Taihu Lake. The present study was conducted at Anhui Research Center for Chinese Alligator Reproduction, namely Chinese Crocodile Lake, an important breeding

center/facility in southern China and plays a vital role in conservation of *A. sinensis*. The Chinese Crocodile Lake was officially opened in 1979, and so far housed over 10,000 captive populations over the past decades and has been achieved concept zoo for tourists.

Fecal sample collection

Fecal samples were collected from sub-adults and adults for examination the cyst of protozoa and eggs of metazoa. Once obtained, the samples were sorted and labeled separately to prevent contamination and mixing. All fecal samples were processed and examined by techniques of physiological saline direct smear, iodine stain, concentrated brine floatation and natural sedimentation, respectively, and were fixed in 4% formalin for preservation and made slides using mounting medium (neutral balsam) for observation.

Necropsy

An autopsy was performed to the alligator for extermination parasites, including digestive system, respiratory system and the muscles of leg. Furthermore, the blood was taken out to examine by direct smear method for haemal parasites, such as hepatozoon. Once the parasite specimens were obtained, washed in PBS, and then fixed in hot 70% ethanol for next observation.

Histological preparation

Tissue samples of *A. sinensis* with parasitism were collected and rinsed in phosphate buffered saline buffer (PBS), and then fixed in 10% formalin overnight, followed by dehydration paraffin embedment. 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.

Results

Intestinal parasite infection status

A total of 148 fecal samples were randomly collected from the captive Chinese alligators living in Anhui breeding centre and underwent final examination. The intestinal parasites finally identified were three protozoa (*Entaoeba* spp., Coccidia), four nematodes (*Ascaris* spp., *Ortleppascaris s*pp., and *Capillaria* spp.) and two trematodes (Schistosomatidae and Cryptogonimidae). In addition, *Ortleppascaris spp.* was identified according to the adult worms in the intestine of captive Chinese alligator. Among which *Ortleppascaris spp.* and *Entaoeba* spp. were the most prevalent and led to the higher infection rate 14.43%, and 9.11%. Interestingly, two or more species of parasite infection were commonly found in one head of a crocodile, and occurrence of parasites was observed in different months or different age of crocodiles.

Autopsy results

A total of eight alligators were dissected, including 7 Chinese alligators with no obvious clinical manifestations and 1 died alligators. We found that 4 Chinese alligators were infected with *Ortleppascaris sinensis* among the 7 Chinese alligators. The number of nematode *O. sinensis* infected are 6, 2 4, 7, respectively. The nematodes are all infected in the stomach and upper intestine of the Chinese alligator. A total of 98 nematode specimens were found in the stomach of the female died Chinese alligator. These nematodes are the same species. Unfortunately, these nematodes were not identified to species. Moreover, we did not find other trematodes in the gastrointestinal tract, trichinella larvae in the muscle, and none *Hepatozoon* in the blood, eventhough we have found some *Hepatozoon* sp. in the blood of snake (Huang NH, 2020). It may be that the sample size is too small, so it is worth further research to expand the size of samples.

Histological findings in the parasitized stomach

Stomachs of the Chinese alligators infected with *O. sinensis* were examined. Gross appearance of the gastric wall 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 was 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 muscular gastric wall appeared thicker and comprised oblique muscle, circular muscle and longitudinal muscle form internal to external. However, there were multifocal superfical 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.

Stomach of the carcas Chinese alligators infected with unidentified nematodes was also examined. On opening the carcass, all visceral organs were found in normal position and appeared grossly normal. Fat deposition was seen in moderate quantities in subcutaneous tissue as well as around visceral organs. Stomach was empty except presence of duckweed, few small stone as well as a little fishbone piece. While gross appearance of the gastric wall infected with unidentified nematodes is prominant pathologic change. The gastric volume is larger, the gastric wall is thinner, gastric plica is flat or disappear and gastric mucosa is pale color. There are extensive, chronic mucosal erosion, necrosis and multifocal ulceration in the whole gastric wall (Fig. 2A). The size and shape of the ulcers were variable, but most were oval and ranged from 2 mm to 185 mm in greatest dimension. The surface of necrosis ulceration covered with yellowish green or dark green effusion (Fig. 2A). We found that the gastric ulcers expanded the submucosa and extended to the muscularis (Fig. 2B). There are even some mucosal necrosis area fused each other. The surface of necrosis covered with a large number of inflammatory exudation and necrotic tissue (Figs 2C and D). In addition, gastric mucosa also revealed presence of numerous spherical to oval, variable sized,

thick-walled and also thin-walled bodies, several of them budding and often forming small chains, sprouting and even germinating, giving rise to branched hyphae (Figs 2D and E). Some granulomatous inflammation (Fig. 2E) and scar structure (Fig. 2F) at the bottom of the lesions were observed, that means clearly ulceration are constructed.

Discussion

In all over the world, information on parasitic infections of crocodiles is richly available (Enabulele *et al.*, 2013; Paperna & Lainson, 2003). Untill today, There are many parasites have been reported from phylum Sarcomastigophora, Heterokontophyta, Apicomplexa, Myxozoa, Platyhelminthes, Rotifera, Acanthocephala, Annelida and Arthropoda infected the alligators and crocodiles (Tellez, 2013). In this study, we also found some cysts of protozoa, eggs of nematodes and trematodes in the fecal samples of Chinese alligators. The adult *O.s sinensis* was acquired through dissecting. The recovery of *O. sinensis* from Chinese alligators in China represents a new geographic record for these parasites and adds to the number of helminths known to infect this crocodile. The parasite community infection crocodiles found in the present study indicated the community structure and diversity of parasites were closely related to such factors as the natural environment, social environment, foods, etc.

Although gastrointestinal nematode infections are considered to be of low or nil pathogenicity, or sub clinical, and not the cause of significant disease (Cubas, 1996), 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 (Huchzermeyer, 2002). Our researches revealed superficial ulcer in the mucous layer and granulomatous inflammation in submucous layer of all gastric walls of Chinese alligators affected by *O. sinensis*. There was a central area of eosinophilic necrosis surrounded by a large number of inflammatory cell infiltration. And there are diffuse mucosal erosion, necrosis and multifocal ulceration in the whole gastric wall in the carcas Chinese alligators infected with unidentified nematodes. Are these nematodes induced the Chinese alligator to die? It is not entirely sure. For the Chinese alligator were found several days after it died, the bacteria group and fungal agent were also conspicuously present in the inflammatory exudation, necrosis area and gastric ulcers tissue under the microscope with HE staining. A diverse array of fungal agents is found in reptiles and amphibians. At least in the present study, the characterization of associated gastric lesions in the current study provides information on associated host injury and parasitological descriptions. In the present case, it is likely that the gastric ulcers of the alligator together with infection of nematodes might have led to low resistance, consequently, this give one favourable for fungi and bacteria to become virulent and cause invasive gastric fungal disease and bacteriosis. The dead reason of the alligator maybe the complex factor arise nematode, bacteria and fungi, while the nematode is possibly the initiate factor.

Conclusions

These findings suggest that the infection of parasites is popular in the captive Chinese

alligators. However, Chinese alligators do not have obvious clinical manifestations, when infected with a small amount of intestinal parasites *O. sinensis*. However, death may be occurring when large numbers of nematodes infected in the gastrointestinal tract of Chinese alligators. This condition calls our great attention to controlling and prevention the parasites in these endangered creatures, though we have achieved in successful breeding of captive Chinese alligators.

Acknowledgements

The author was grateful to Professor Xiaobing Wu and Fang Zhang from Anhui Normal University, Dr. Marisa Tellez from University of California, and Professor Bing Huang from Shanghai Institute of Veterinary Research, Chinese Academy of Agricultural Science, for assistance in identifying eggs from fecal samples and encouragement in the process of experiment. I greatly appreciate the Chinese alligator raisers for providing guidance and technical assistance during our sample collection.

References

Cubas, Z.S. (1996) Special challenges of maintaining wild animals in captivity in South America. *Rev Sci Tech,* **15**, 267-287. doi: 10.20506/rst.15.1.918.

Enabulele, E.E., Ozemoka, H.J., Awharitoma, A.O. & Aisien, M.S. (2013) Parasitic infections of the African dwarf crocodile (Osteolaemus tetraspis) and the ornate Nile monitor (Varanus ornatus) from Nigeria. *Acta Parasitol,* **58**, 191-197. doi: 10.2478/s11686-013-0131-0.

Huang NH, W.Y., Li YY, Zhao JH (2020) A new record of Hepatozoon sp. Miller, 1908 (Apicomplexa: Adelerina) infected in the snake Naja atra (Squamata: Dipsadidae) in China. *Pakistan Journal of Zoology*.

Huchzermeyer, F.W. (2002) Diseases of farmed crocodiles and ostriches. *Rev Sci Tech,* **21**, 265-276. doi: 10.20506/rst.21.2.1334.

Leclaire, S. & Faulkner, C.T. (2014) Gastrointestinal parasites in relation to host traits and group factors in wild meerkats Suricata suricatta. *Parasitology,* **141**, 925-933. doi: 10.1017/S0031182013002333.

Magnino, S., Colin, P., Dei-Cas, E., Madsen, M., McLauchlin, J., Nockler, K., Maradona, M.P., Tsigarida, E., Vanopdenbosch, E. & Van Peteghem, C. (2009) Biological risks associated with consumption of reptile products. *Int J Food Microbiol,* **134**, 163-175. doi: 10.1016/j.ijfoodmicro.2009.07.001.

Paperna, I. & Lainson, R. (2003) Ultrastructural studies on the sporogony of Hepatozoon

spp. in Culex quinquefasciatus say, 1823 fed on infected Caiman crocodilus and Boa constrictor from northern Brazil. *Parasitology,* **127**, 147-154. doi: 10.1017/s0031182003003482.

Tellez, C.M. (2013) *A checklist of host-parasite interactions of the order crocodylia*. Berkeley, CA, University of California Press.

Wang Q, H.D., Yang GY, Jiang B, Niu LL, Zhao B. (2007) Investigation on parasitic infection in reptiles at a zoo in Chengdu. *Sichuan J Zool*.

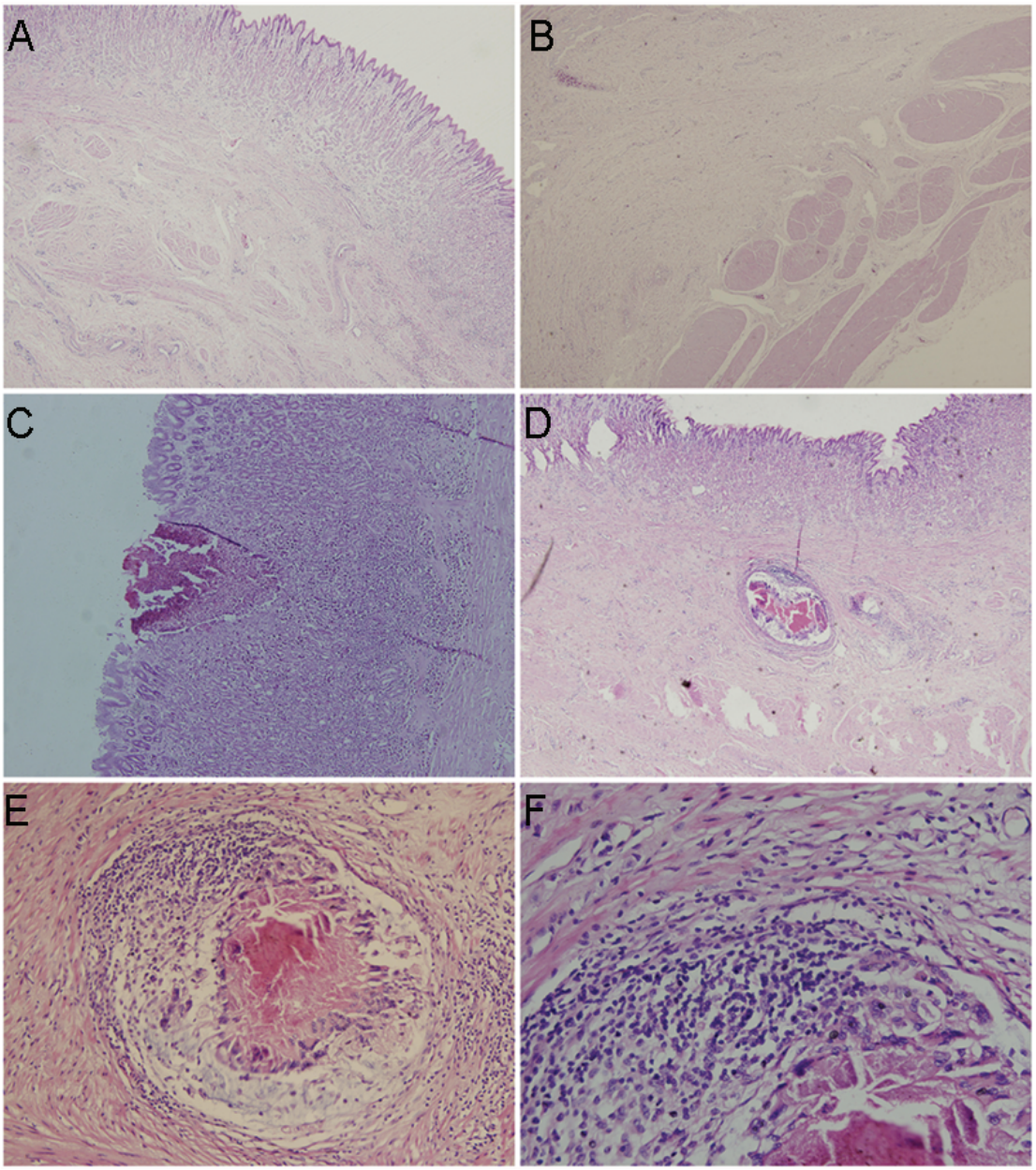


Fig. 1 Observation of gastric wall tissue sections (HE staining). (A) Normal (mucosa and submucosa layer, ×40); (B) Normal (submucosa and muscular layer, ×40); (C) Superficial erosions (mucosa layer, ×100); (D) Ggranulomatous inflammation (mucosa and submucosa layer, ×40); (E) Granulomatous inflammation (submucosa layer, ×200); (F) Granulomatous inflammation (submucosa layer, ×400)

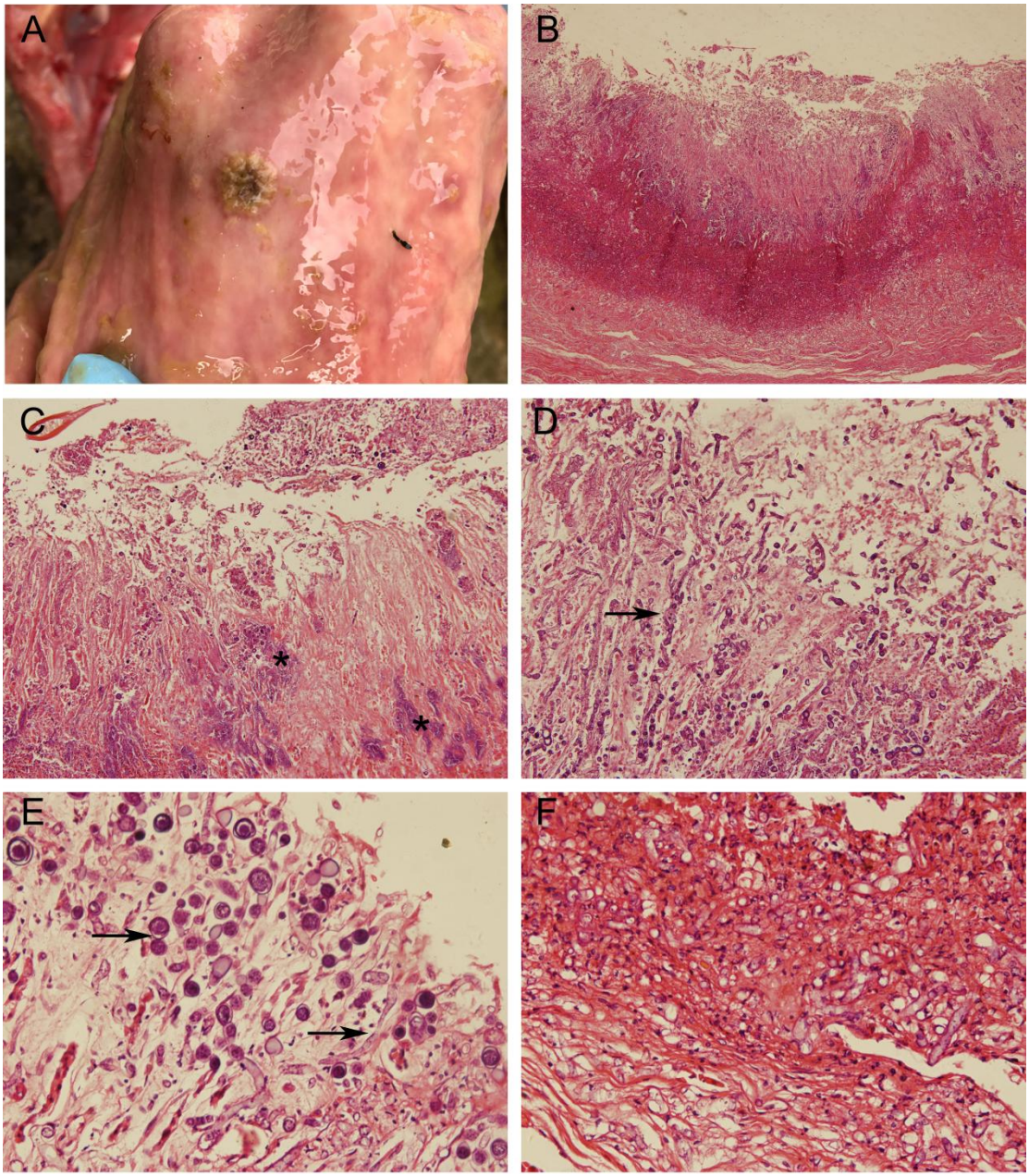


Fig. 2. Stomach of the Chinese Alligator infected with nematodes. (A) Multifocally extensive, chronic gastric ulcers are shown. (gross appearance); (B) Gastric ulcers involved the mucosa, submucosa, muscular layer (HE staining, ×40); (C) Inflammatory exudation and necrotic tissue. There are a lot of bacteria group in necrotic area (*) (HE staining, ×100); (D) Inflammatory exudation and necrotic tissue. There are also a lot of hyphae and spores of fungi. (arrows) (HE staining, ×100); (E) Granulomatous tissue. There are also a lot of hyphae and spores of fungi (arrows) (HE staining, ×400); (F) scar structure tissue (HE staining, ×400).