



Original/Investigación animal

Preliminary report on the intestinal parasites and their diversity in captive chinese alligators

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Abstract

Objective: Although intestinal parasites are commonly detected in either wild or farmed crocodiles in other parts of the world, yet few reports have ever been on the captive bred Chinese alligators (*Alligator sinensis*) since they are only distributed in the southern areas of the Yangtze River. The current work was undertaken to investigate the intestinal parasites and their diversity in the Chinese crocodilian species.

Methods: In May and October of 2013, we randomly collected a total of 328 fecal samples of the captive Chinese alligators in a breeding centre in south Anhui province.

Results: Three genera of protozoa (*Entamoeba*, *Eimeria* and *Isospora*), five genera of nematodes (*Ascaris*, *Dujardinascaris*, *Capillaria*, *Toxocara* and *Strongylus*), two genera of trematodes (*Echinostoma* and *Clonorchis*) and two families of trematodes (*Schistosomatidae* and *Cryptogonimidae*) were identified, in which *Dujardinascaris* was the most prevalent and led to the highest infection rate (14.33%), and *Entamoeba* ranked the second (13.11%). The number of species, richness index, diversity index and evenness index were higher in July and August, and those indexes were relatively most in juvenile and sub-adult alligators.

Conclusions: Our findings suggest that the intestinal parasites infection was prevalent in captive Chinese alligators, and this condition requires our attention, whatever it is for controlling or preventing the intestinal parasitic disease from spreading to humans or conservation of this endangered species.

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Key words: *Intestinal parasites*. *Chinese alligators*. *Diversity*. *Anhui province*.

INFORME PRELIMINAR SOBRE LOS PARÁSITOS INTESTINALES Y SU DIVERSIDAD EN CAIMANES CHINOS EN CAUTIVIDAD

Resumen

Objetivo: Aunque los parásitos intestinales son comúnmente detectados en los cocodrilos salvajes o en criados en cautividad en otras partes del mundo, existen muy pocos informes el caso concreto del caimán chino criado en cautividad (*Alligator sinensis*), ya que sólo están distribuidos en las zonas del sur del río Yangtze. El presente trabajo fue realizado para investigar los parásitos intestinales y su diversidad en las especies de cocodrilo chino.

Métodos: En mayo y octubre de 2013, se recopilieron aleatoriamente un total de 328 muestras fecales de caimanes chinos en cautividad de un centro de cría en el sur de la provincia de Anhui.

Resultados: Fueron identificados tres géneros de protozoos (*Entamoeba*, *Eimeria* e *Isospora*), cinco géneros de nematodos (*Ascaris*, *Dujardin Ascariis*, *Capillaria*, *Toxocara* y *Strongylus*), dos géneros de trematodos (*Echinostoma* y *Clonorchis*) y dos familias de trematodos (*Schistosomatidae* y *Cryptogonimidae*), de los cuales la mayor prevalencia correspondió a *Dujardin Ascariis* y producían la mayor tasa de infección (14,33%); *Entamoeba* se situó en el segundo puesto (13,11%). El número de especies, índice de riqueza, índice de diversidad y de equitatividad fue superior en los meses de julio y agosto, y éstos, relativamente más para caimanes jóvenes y sub-adultos.

Conclusiones: Nuestros resultados apuntan la prevalencia de la infección por parásitos intestinales en caimanes chinos en cautividad y ello exige nuestra atención, ya sea para controlar o prevenir la propagación de la enfermedad parasitaria intestinal a los seres humanos, o para la conservación de esta especie en peligro de extinción.

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Palabras clave: *Parásitos intestinales*. *Caimán chino*. *Diversidad*. *Provincia de Anhui*.

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Introduction

Animal parasitic disease has characteristics of easy infection and transmission diversity, and makes it to become the major disease to wildlife^{1,2}. Crocodiles, in common with other reptiles, usually harbor a wide variety of protozoa and metazoans external and internal parasites³. In other parts of the world, information on the parasitic infections of crocodiles is richly available³⁻⁶. There revealed the presence of eight helminthes (*Acanthostomum americanum*, *Pelaezia loossi*, *Telorchis* sp. juv., *Pseudoneodiplostomum groschaffi* sp. n., *Dujardinascaris helicina*, *Contraecaeum* sp. Type 2 larvae, *Micropleura* sp. and *Paratrichosoma recurvum*) in the Morelet's crocodile from the Lagoon of Celestún, Yucatan, Mexico^{7,8}. One species of pentastome (*Sebekia oxycephala*), two species of nematodes (*Dujardinascaris waltoni* and *Multicaecum tenuicolle*), four species of trematodes (*Polycotyle ornata*, *Acanthostomum coronarium*, *Archaeodiplostomum acetabulatum* and *Pseudocrocodilicola americanense*) and one species of hemogregarine (*Haemogregarina crocodilnorum*) were recovered in American alligators from South Carolina⁹. There investigated the parasite infection of *Alligator mississippiensis* from 3 lakes in north-central Florida using a gastric lavage technique, and found four nematodes (*Dujardinascaris waltoni*, *Ortleppascaris antipini*, *Brevimulticaecum tenuicolle* and larvae of *Contraecaeum* sp.)¹⁰.

The Chinese alligator, *Alligator sinensis* Fauvel, 1879, a unique animal in China, is listed as a critically endangered species. Although recent progress has been made to ensure the species survival by captive-born means, yet there go threats from physique angular, loss of appetite, and a large number of death caused by digestive tract parasitizing of the nematodes¹¹ or other artificial factors. Nevertheless, little is known about the parasitic fauna infecting the Chinese alligators, and such reports are rare, either. The only information in the literature regarding the occurrence of parasite infection in crocodiles is available from Wang *et al.* (2007)¹², 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.

Unfortunately, little attention has ever paid on the follow-up study, even if the intestinal parasites are of great importance for the endangered Chinese alligator itself or the health of raisers in the breeding center. In order to understand the prevalence of the intestinal parasites in this species, we collected the fecal samples of Chinese alligators between May and October of 2013 in a captive breeding center in south Anhui province to examine the parasites and eggs as well as the numerical characteristics of parasite community, with an attempt to provide evidence for controlling the parasitic diseases in this species population and the safety of the feeders in breeding center.

Methods

Study site

Chinese alligator, one of the known living species of alligator, 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 tourists¹³.

Fecal sample collection and examination

Collection of the fecal samples was carried out on every 15th day of the month from May to October in 2013, and the samples were sorted out from yearlings, juveniles, sub-adults and adults. Because the research centre is located in the subtropical humid monsoon climate zone, the Chinese alligator stays dormant from November to April the following year. Sample collection was performed in the morning under the assistance of the raisers. 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.

Data processing

1. The Margalef's richness index (R) was used to measure the biodiversity¹⁴, where R represents the quantity of species within a community. The Margalef index was calculated from the equation $R=(S-1)/\ln N$, where S indicates the total number of species recorded, and N represents the total number of individuals in the sample.
2. The Pielou's evenness index (J) was used to measure the biodiversity¹⁵. The species evenness index was not positively relevant to species richness index, where J represents the evenness extent of species distribution instead of the quantity of species recorded. The Pielou index was calculated as $J=H/\ln S$, where S denotes the total number of species in the community and H means the Shannon-Wiener diversity index.
3. The Shannon-Wiener's diversity index (H) was used to measure the biodiversity¹⁶, for it can estimate the evenness with which individuals of the

community are divided among the taxon present, particularly, for a given equitability, the index increases either as the species richness increases or species diversity increases. The Shannon-Wiener's index calculated as $H = -\sum P_i \ln P_i$, where P_i denotes the proportion of individuals in the total species.

- The Simpson's dominance index (D) was introduced to measure the biodiversity¹⁷. Simpson's index is heavily weighted towards the most abundant species in the sample, while being less sensitive to species richness. As D increases, diversity decreases, too. The Simpson's index was calculated by equation of $D = \sum P_i^2$.

Results

Intestinal parasite infection status in captive Chinese alligators

A total of 328 faecal 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 (*Entamoeba* spp., *Eimeria* spp. and *Isospora* spp), five nematodes (*Ascaris* spp., *Dujardinascaris* spp., *Capillaria* spp., *Toxocara* spp. and *Strongylus* spp.) and four trematodes (*Echinostoma* spp., *Clonorchis* spp., Schistosomatidae and Cryptogonimidae). In addition, *Dujardinascaris* spp. was identified according to the adult worms in the intestine of captive Chinese alligator, yet we failed to identify the genera for Schistosomatidae and Cryptogonimidae. 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.

The number of samples collected in May, June, July, August, September and October were 61, 72, 58, 61, 58 and 18, respectively, and most captive Chinese alligators were infected with at least one intestinal parasite, especially in July and August. Meanwhile, it was found that highest occurrence compared to other intestinal parasites were *Dujardinascaris* spp. (14.3%), followed by *Entamoeba* spp. (13.11%). *Entamoeba* spp. occurred most in May (6.56%) and June (11.10%), *Dujardinascaris* spp. and *Entamoeba* spp. occurred most in July, August and September (27.60%, 24.60% and 15.50% vs. 20.70%, 21.30% and 10.34%, respectively), whereas the most species in October was *Ascaris* spp. (11.10%) (Table I), and *Dujardinascaris* spp. were spatially distributed in different months in a typical year (Table I).

The number of samples collected from alligators of yearlings, juveniles, sub-adults and adults were 86, 88, 76 and 78, respectively. The highest occurrence in yearling, juvenile and sub-adult was involved in all *Entamoeba* spp. by 10.5%, 14.80% and 13.20%, respectively. However, *Dujardinascaris* spp. occurred most in adults was 41.00% (Table II). *Entamoeba* spp., *Eimeria* spp., *Dujardinascaris* spp., Schistosomatidae and Cryptogonimidae were spatially distributed in different years of life of alligators (Table II).

Intestinal parasite infection diversity for captive Chinese alligators in different months

The parasite species were most abundant in July and August (12 species) and least in October (2 species).

Parasites		May	June	July	August	September	October	Total
		(%)*	(%)*	(%)*	(%)*	(%)*	(%)*	(%)**
Protozoan	<i>Entamoeba</i> spp.	6.56(4/61)	11.11(8/72)	20.69(12/58)	21.31(13/61)	10.34(6/58)	0	13.11(43/328)
	<i>Eimeria</i> spp.	1.64(1/61)	2.78(2/72)	13.80(8/58)	9.84(6/61)	0	0	5.18(17/328)
	<i>Isospora</i> spp.	0	1.39(1/72)	6.90(4/58)	9.84(6/61)	3.45(2/58)	0	3.96(13/328)
Nematode	<i>Ascaris</i> spp.	0	1.39(1/72)	10.30(6/58)	6.56(4/61)	8.62(5/58)	11.10(2/18)	5.49(18/328)
	<i>Dujardinascaris</i> spp.	3.28(2/61)	5.56(4/72)	27.60(16/58)	24.60(15/61)	15.50(9/58)	5.56(1/18)	14.3(47/328)
	<i>Capillaria</i> spp.	0	2.78(2/72)	8.62(5/58)	3.28(2/61)	0	0	2.74(9/328)
	<i>Toxocara</i> spp.	0	0	6.90(4/58)	9.84(6/61)	6.90(4/58)	0	4.27(14/328)
	<i>Strongylus</i> spp.	0	1.39(1/72)	12.10(7/58)	9.84(6/61)	0	0	4.27(14/328)
Trematode	<i>Echinostoma</i> spp.	1.64(1/61)	0	10.30(6/58)	8.20(5/61)	1.72(1/58)	0	3.96(13/328)
	<i>Clonorchis</i> spp.	0	0	6.90(4/58)	4.92(3/61)	3.45(2/58)	0	2.74(9/328)
	Schistosomatidae	0	1.39(1/72)	5.17(3/58)	6.56(4/61)	1.72(1/58)	0	2.74(9/328)
	Cryptogonimidae	0	5.56(4/72)	6.90(4/58)	8.20(5/61)	3.45(2/58)	0	4.57(15/328)

* Infection rate is positive samples to total samples in different months. **Infection rate is positive samples to total samples in all months.

Table II
Infection rate of intestinal parasite infection for captive Chinese alligators in different years of life

Parasites		Yearlings	Juveniles	Sub-adults	Adults	Total
		(%)*	(%)*	(%)*	(%)*	(%)**
Protozoan	<i>Entamoeba</i> spp.	10.5(9/86)	14.80(13/88)	13.20(10/76)	14.10(11/78)	13.11(43/328)
	<i>Eimeria</i> spp.	2.33(2/86)	3.41(3/88)	10.50(8/76)	5.13(4/78)	5.18(17/328)
	<i>Isospora</i> spp.	4.65(4/86)	3.41(3/88)	7.89(6/76)	0	3.96(13/328)
Nematode	<i>Ascaris</i> spp.	0	4.55(4/88)	10.50(8/76)	7.69(6/78)	5.49(18/328)
	<i>Dujardinascaris</i> spp.	4.65(9/86)	5.68(5/88)	7.89(6/76)	41.00(32/78)	14.3(47/328)
	<i>Capillaria</i> spp.	3.49(3/86)	1.14(1/88)	6.58(5/76)	0	2.74(9/328)
	<i>Toxocara</i> spp.	0	4.55(4/88)	7.89(6/76)	5.13(4/78)	4.27(14/328)
	<i>Strongylus</i> spp.	0	3.41(3/88)	9.21(7/76)	5.13(4/78)	4.27(14/328)
Trematode	<i>Echinostoma</i> spp.	2.33(2/86)	3.41(3/88)	5.26(4/76)	5.13(4/78)	3.96(13/328)
	<i>Clonorchis</i> spp.	2.33(2/86)	4.55(4/88)	0	3.85(3/78)	2.74(9/328)
	Schistosomatidae	0	4.55(4/88)	2.63(2/76)	3.85(3/78)	2.74(9/328)
	Cryptogonimidae	4.65(4/86)	5.68(5/88)	6.58(5/76)	1.28(1/78)	4.57(15/328)

* Infection rate is positive samples to total samples in different years Chinese alligators. **Infection rate is positive samples to total samples in all Chinese alligators.

The richness index was topmost in August (2.55), sequentially followed by June (2.52), July (2.52), September (2.31), May (1.44) and October (0.91). The greatest value of diversity index was 2.35 for July and 2.33 for August compared to the smallest (0.64) in October. The value of evenness index differed somewhat in different months, and the largest value of dominance index was 0.56 for October and the smallest was 0.11 and 0.11 respectively for July and August (Table III).

Intestinal parasite infection diversity for captive Chinese alligators in different years of life

The juveniles were mostly affected by diverse intestinal parasites (12 species), and the yearling did relatively less (8 species). The richness index stood highest in the juveniles (2.78), secondarily in sub-adults

(2.38), adults (2.10) and yearlings (2.06). The diversity index ranked from 2.34 for sub-adults, 2.32 for the juvenile, 1.94 for yearlings to 1.82 for adults. The evenness index was maximal in sub-adults (0.97) and minimal in adults (0.79). Value of dominance index was as high as 0.24 in adults and remained comparatively smaller in sub-adults (0.10) (Table IV).

Discussion

The majority of the captive Chinese alligators examined in current study were infected with at least one intestinal parasite species, and most of these parasitic species are recorded previously in diverse species of crocodiles except *Clonorchis*, for some trematodes belonging to *Clonorchis* is endemic to China, which suggests that parasitic infection with crocodil-

Table III
Diversity of intestinal parasite infection for captive Chinese alligators in different months

Month	Number of species	Number of individuals	Richness index	Diversity index	Evenness index	Dominance index
	S	N	R	H	J	D
May	4	8	1.44	1.21	0.88	0.34
June	9	24	2.52	1.91	0.87	0.19
July	12	79	2.52	2.35	0.95	0.11
August	12	75	2.55	2.33	0.94	0.11
September	9	32	2.31	1.96	0.89	0.17
October	2	3	0.91	0.64	0.92	0.56

Table IV
Diversity of intestinal parasite infection for captive Chinese alligators in different years of life

Years	Number of species	Number of individuals	Richness index	Diversity index	Evenness index	Dominance index
	<i>S</i>	<i>N</i>	<i>R</i>	<i>H</i>	<i>J</i>	<i>D</i>
Yearlings	8	30	2.06	1.94	0.93	0.17
Juveniles	12	52	2.78	2.32	0.93	0.12
Sub-adults	11	67	2.38	2.34	0.97	0.10
Adults	10	72	2.10	1.82	0.79	0.24

ian is characterized to a certain extent by geographic distribution. Importantly, some parasites identified in our work are known to be pathogenic to both animals and human (animal handlers and visitors)¹⁸.

The intestinal parasite species we identified consist of protozoa in genera *Entamoeba* (Lobosea), *Eimeria* (Coccidia) and *Isospora* (Coccidia). They were previously reported in crocodilians^{19,20}. Crocodilians were considered to be resistant carriers of this parasite *Entamoeba*²¹. However, coccidia may become pathogenic, causing illness or death in younger alligators under captive feeding and reproduction¹⁹. Buenviaje *et al.*²² described coccidiosis as one of the major disease affecting farmed crocodiles in Queensland and the Northern Territory, yet the infection rate of *Eimeria* (5.18%) and *Isospora* (3.96%) was relatively lower in our findings. *Entamoeba* appeared the second higher infection rate (13.11%) in all parasite species, but affected dominantly the host in different seasons or different ages of crocodiles. These findings showed that the amoebic pollution was serious in the breeding centre.

Five nematodes in genera *Ascaris*, *Capillaria*, *Strongylus*, *Dujardinascaris* and *Toxocara* were identified, and all the nematode have been recoded in other crocodiles, such as the eggs of *Ascaris lumbricoides* were found in the fecal samples of a zoo-kept *Crocodylus catapraectus*²³, *Capillaria* can be acquired from crocodiles in Australia^{22,24}, Papua New Guinea¹⁹ and Irian Jaya²⁵, *Strongylus sp.* was acquired from the intestine of *Alligator mississippiensis* in New York Aquarium, New York, USA, by Biosystematics and the US National Parasite Collection (NPC) in 2010, *Dujardinascaris* and *Toxocarawere* also reported in previous literature for other crocodiles^{8,10,26,6}. In this study, we found that *Dujardinascaris* was the most prevalent species in Anhui breeding centre, and led to the highest infection rate (14.33%), especially in July and August. It is also a major parasitic species in adult captive Chinese alligators, a similar finding in north-central Florid¹⁰, where the *Dujardinascaris waltoni* was recognized as the most prevalent species in all 3 lakes (Apopka, Griffin and Woodruff). Again, in Okavango River, Botswana, *Dujardinascaris madagascariensis* was found to have mostly infected the

host *Crocodylus niloticus*²⁶. Gastrointestinal nematode infection in crocodiles is often asymptomatic, but which may be occasionally associated with the disease, such as gastric ulceration and runting in hatchlings with infection of *Dujardinascaris*¹⁹.

We also found trematodes are composed of *Echinostoma* spp., *Clonorchis* spp, Schistosomatidae and Cryptogonimidae. However, the total infection rate of each trematode was not high (2.74%-4.57%). And some eggs were identified to family Schistosomatidae and Cryptogonimidae only, for there was a very similar in morphology about these eggs. The *Echinostoma* sp. was recorded from intestine of *melanosuchus niger* in Mato Grosso, Brazil²⁷, yet *Clonorchis* are the first time recorded in captive Chinese alligators, for some trematode belonging to *Clonorchis* is endemic to China. Schistosomatidae in genera *Griphobilharziawas* recorded in crocodiles²⁵. The family Cryptogonimidae recorded is associated with the genera of *Acanthostomum*, *Caimanicola*, *Capsulodiplostomum*, *Proctocaecum* and *Timoniella*^{8,9,28,29}. These parasites were not considered to be the cause of significant disease in their crocodilian hosts. But, *Acanthostomum loossi* recovered from farmed *Crocodylus acutus* and *Crocodylus rhombifer* in Cuba was associated with poor health and growth rates³⁰.

The richness index, diversity index, evenness index and dominance index of community diversity were often analysis in order to compare them with the diversity found in different community, such as in vegetation, birds, soil animals, bacteria and mites, etc.^{31,32,33}. Nevertheless, no parasitic diversity data regarding the parasites infection crocodiles were present. Our measurement on the indices in different months showed the highest parasitic diversity (richness index $R=2.55$, diversity index $H=2.35$, evenness index $J=0.95$, Table III) in July or August over the remaining months, in which October was the lowest ($H=0.64$, $R=0.91$, Table III), and the Simpson's dominance index appeared totally inverse. This may be explained that the environment of the breeding centre, a region in sub-tropical monsoon climate with warm temperature and humidity, plenty of rainfall and distinct four seasons. July and August are the summer season that is favorable to the parasite growth and

reproduction. However, the parasitic infection is less in October, for it turns cold, and Chinese alligator begins to go into hibernation. On examination of the different years of alligators, the juvenile had maximal richness value ($R=2.78$), sub-adults had the highest values of diversity ($H=2.34$) and evenness ($J=0.97$). This may attributed to the juvenile and sub-adults were breeding in the same area with similar breeding conditions that were in relatively serious parasitic pollution. Contrarily, smallest number of parasite species (8 species) and lower richness index ($R=2.06$) were found in the yearling, for they were bred in strictly treated food and clean environment and less human interference. Meanwhile, the fecal samples of adult crocodiles were collected from a relatively wider area with rich variety of food in the breeding centre, but showed lowest values of diversity index ($H=1.82$) and evenness index ($J=0.79$) and highest values of dominance index ($D=0.24$). This also tells us that the parasitic community infection in adult alligators is mainly composed of several parasites, which provides guidance for prevention of parasitic disease. 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.

Conclusion

This is the first report on the parasitic infection in the captive Chinese alligator in a breeding center in south Anhui province. The findings suggest that the infection of parasites is popular, even if the alligators are bred in a closed area. Besides, the number of species, richness index, diversity index and evenness index are higher in July and August, and the juvenile and sub-adults appear mostly affected. 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.

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Author disclosure statement

The authors have no conflicts of interest.

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