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Investigation of giardiasis in captive animals in zoological gardens with strain typing of assemblages in China

Hua Liu[1,*], Bin Wang[2,3,*], Jianhai Yin[1], Zhongying Yuan[1], Yanyan Jiang[1], Jing Zhang[1], Jianping Cao[1], Yujuan Shen[1] and Hui Liu[2]

[1]National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Chinese Center for Tropical Diseases Research), NHC Key Laboratory of Parasite and Vector Biology, WHO Collaborating Centre for Tropical Diseases, National Center for International Research on Tropical Diseases, Shanghai 200025, China; [2]Department of Parasitology, Zunyi Medical University, Zunyi 563000, China and [3]Guizhou Center for Disease Control and Prevention, Guiyan 550000, China

Research Article

*These authors contributed equally to this work.

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Authors for correspondence:
Yujuan Shen, E-mail: shenyj@nipd.chinacdc.cn; Hui Liu, E-mail: liuhui6032@sina.com

Abstract

Giardia duodenalis is a common zoonotic intestinal pathogen. It has been increasingly reported in humans and animals; however, genotyping information for *G. duodenalis* in captive animals is still limited. This study was conducted to assess the prevalence and multilocus genotyping of *G. duodenalis* in captive animals in zoological gardens in Shanghai, China. A total of 678 fresh fecal samples were randomly collected from captive animals including non-human primates (NHPs) ($n = 190$), herbivores ($n = 190$), carnivores ($n = 151$), birds ($n = 138$) and reptiles ($n = 9$) in a zoo and were examined for the presence of *G. duodenalis* using nested polymerase chain reaction (nested PCR). All *G. duodenalis* positive samples were assayed with PCR followed by sequencing at *β*-giardin (*bg*), glutamate dehydrogenase (*gdh*) and triose phosphate isomerase (*tpi*) genes. In this study, 42 specimens (6.2%) were tested *G. duodenalis*-positive of the 678 fecal samples examined based on a single locus. A total of 30 (4.4%), 30 (4.4%) and 22 (3.2%) specimens were successfully amplified and sequenced at *gdh*, *tpi* and *bg* loci, respectively. Assemblages A and B were identified with assemblage B dominating in NHPs. Sequence analysis demonstrated that one, two and five new isolates were identified at *bg*, *gdh* and *tpi* loci. DNA sequences and new assemblage-subtypes of zoonotic *G. duodenalis* assemblages A and B were identified in the current study. Our data indicate the occurrence and molecular diversity of *G. duodenalis* and the potential zoonotic transmission in captive animals in China.

Introduction

Giardia duodenalis (syns *G. lamblia*, *G. intestinalis*) is one of the most common enteric parasites worldwide with a diverse range of hosts that include humans, wildlife, domestic and companion animals (Feng *et al.*, 2011). *Giardia duodenalis* is the only species found in humans and being increasingly recognized to cause gastrointestinal infection ranging from asymptomatic to severe diarrhoea as well as chronic diseases and malabsorption (Halliez *et al.*, 2013). *Giardia* is mainly transmitted through the ingestion of cysts in contaminated surface food or water and direct contact with infected individuals, or directly *via* the fecal/oral route (Cacciò *et al.*, 2008). *Giardia duodenalis* is now considered to be divided into at least eight distinct genetic assemblages (A to H), of which assemblages A and B have the broadest host ranges and are mainly found to infect humans and other various mammals. The remaining assemblages (C to H) have strong host specificities and narrow host ranges. Assemblages C and D, for example, have been found in domestic dogs and other canines. Assemblage E has been found largely in domestic ruminants and pigs. Assemblages F and G are specific to cats and livestock, respectively, whereas assemblage H is usually found in marine vertebrates (Feng *et al.*, 2011; Ryan *et al.*, 2013).

In recent years, the number of people visiting zoos has increased and people also like to be in contact with animals with the new construction of wildlife parks. Thus, the risk of potential zoonosis will increase and the role of captive animals, particularly in big cities, should be taken into consideration. Moreover, a growing number of emerging wildlife infectious diseases have posed a risk to human health and the investigations of wildlife diseases have been recognized as an important part of global health (Daszak *et al.*, 2000). *Giardia* have been reported in captive animals such as non-human primates (NHPs) and wild birds worldwide, and zoonotic assemblages A and B of *G. duodenalis* have been found, which indicates the importance of the study of *G. duodenalis* in wild animals (Oates *et al.*, 2012; Reboredo *et al.*, 2015; Mynářová *et al.*, 2016). Animal husbandry is commonplace in China and the role of livestock and pets in the transmission of Giardia has been studied extensively (Liu *et al.*, 2014, 2017; Qi *et al.*, 2016). However, very little information is available on the prevalence of *G. duodenalis* in the captive animals in China, and the assemblage distribution and the multilocus genotyping (MLG) of *G. duodenalis* remain unclear.

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Table 1. Infection rates based on *gdh*, *tpi* and *bg* loci used by nested PCR

Animal species	Number	Positive samples	Infection rate (%)	*gdh*-positive	*tpi*-positive	*bg*-positive
NHPs	190	33	17.3	24	23	19
Carnivores	142	4	2.8	2	4	2
Herbivores	190	2	1.0	1	2	0
Birds	138	2	1.4	2	1	1
Reptiles	9	0	0	0	0	0
Others	9	1	1.1	1	0	0
Total	678	42	6.2	30	30	22

Therefore, the study was conducted to assess the prevalence and genetic characteristic description of zoonotic *G. duodenalis* in animals housed within Shanghai Wild Animal Zoo. The fecal samples from different animals were collected and examined the presence of *G. duodenalis* using nested polymerase chain reaction (nested PCR). In addition, the *G. duodenalis* genotypes were elucidated based on the common target genes: glutamate dehydrogenase (*gdh*), triose phosphate isomerase (*tpi*) and *β*-giardin (*bg*) (Feng *et al.*, 2011). Then the potential for zoonotic transmission of *G. duodenalis* in these animals was assessed at the three loci.

Methods

Study area and sample collection

A total of 678 fecal specimens were collected from captive animals in a zoo in Shanghai, including NHPs ($n = 190$), herbivores ($n = 190$), carnivores ($n = 151$), birds ($n = 138$) and reptiles ($n = 9$). Each fresh fecal specimen was obtained using a disposable glove and plastic container. All the specimens were transported to NHC Key Laboratory of Parasite and Vector Biology of NIPD in a cooler with ice packs within 24 h of collection and processed as previously described (Fayer *et al.*, 2000) and were maintained at 4°C for no more than 3 days before DNA extraction.

DNA extraction and PCR amplification

Genomic DNA was extracted from all fecal samples using the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, USA) following the manufacturer's instructions. Purified DNA samples (200 μL) were stored at −20°C for further molecular analysis. To determine *G. duodenalis* subtypes, *gdh*, *tpi* and *bg* genes were simultaneously amplified and the PCR amplification parameters have been previously described (Sulaiman *et al.*, 2003; Lalle *et al.*, 2005; Cacciò *et al.*, 2008). In all the PCR reactions, a dog-derived *Giardia*-positive DNA specimen (assemblage C) and distilled water were used as positive and negative controls, respectively. The second PCR products were analysed using 1.5% agarose gel stained with ethidium bromide.

Sequence and genotype analysis

All target fragments were amplified at least three times and all the PCR-positive products were sequenced in both directions using the secondary primers on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, USA) using a Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) (Long *et al.*, 2018). Nucleotide sequences obtained in this study were aligned, examined and compared with reference sequences downloaded from the GenBank database using Clustal X 1.83 and Basic Local Alignment Search Tool (BLAST).

Accession numbers of the nucleotide sequences

The new sequences generated in this study have been deposited in the GenBank under accession numbers MT811038–MT811045.

Results

Prevalence of G. duodenalis by PCR

Of the 678 fecal samples examined, 42 (6.2%) were tested *G. duodenalis*-positive at least based on a single locus. A total of 30 (4.4%), 30 (4.4%) and 22 (3.2%) specimens were successfully amplified and sequenced at *gdh*, *tpi* and *bg* loci, respectively. MLG data at the three loci tested were available for 14 samples and no mixed infections were found. In addition, 12 and 16 *G. duodenalis* isolates were successfully amplified at two and one loci, respectively.

Different animals have different infection rates; NHPs have the highest infection rate reaching 17.4% (33/190). Of the 33 positive isolates, 14 *Lemur catta*, 8 *Pantroglodytes*, 7 *Lemur variegatus*, 2 *Rhinopithecus* and 2 *Saimiri sciureus* were obtained for *G. duodenalis*. Following by, the infection rate of carnivores was 2.8% (4/142), with four *Helarctos malayanus* being identified. The infection rate of birds was 1.4% (2/138) including one *Dromaius novaehollandia* and one *Acridotheres cristatellus*, and 1.1% (2/190) of herbivore animals with one *Oryx* and one *Damaliscus dorcas* being identified. One *Myrmecophaga tridactyla* was also being identified to be *Giardia*-positive. No giardia infection was found in reptiles (Table 1).

Molecular genotyping at the bg gene

Amplification of a 510 fragment of the *bg* gene was obtained from 22 of 42 (52.38%) *G. duodenalis* isolates. At *bg* gene, 19 sequences were identified as assemblage B and three were assemblage A. In addition, sequence analysis revealed one (SHWD185) and three (SHWD159, SHWD168 and SHWD357) isolates and have 100% similarity with the human-derived isolates MG746614 and MG736242, respectively. A new isolate (GenBank no. MT811038) was identified at *bg* locus with one base mutation (A→G) with the reference sequence KJ88974 in the GenBank.

Molecular genotyping at the gdh gene

At 30 *gdh*-positive specimens, assemblages A and B were found in 3 (10%) and 27 (90%) specimens, respectively, with assemblage B being the major genotypes (Table 2). Two new sequences were identified in NHPs, one sequence (GenBank no. MT811041) has two base differences (G→A, G→A) and the other sequence (MT811040) also has two base mutations (C→T, C→T) compared with the deposited sequence in the GenBank (KM977648) (see Supplementary Table S2).

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Table 2. Characterization of *Giardia duodenalis* based on multi-loci of *gdh*, *tpi* and *bg* genes

			Genotype		
Animal species	Latin name	Sample number	*gdh*	*tpi*	*bg*
NHPs					
Hominidae	*Pantroglodytes* (8)	2	B	–	B
		16	B	B	B
		17	B	B	B
		18	B	B	–
		20	B	–	–
		316	B	–	–
		319	B	–	–
		185	B	B	B
Lemuridae	*Lemur catta* (14)	12	–	B	B
		14	B	B	B
		15	B	B	B
		25	B	B	B
		53	–	B	B
		359	–	B	B
		360	–	B	–
		362	B	–	B
		363	B	B	–
		365	B	B	B
		366	B	B	B
		367	B	–	–
		370	B	B	B
		57	–	B	–
	Lemur variegatus (7)	159	–	B	B
		160	–	B	–
		162	B	–	B
		164	B	B	–
		166	–	B	–
		167	–	B	–
		168	B	B	B
Cercopithecidae	*Rhinopithecus* (2)	343	B	–	–
		357	B	B	B
Cebidae	*Saimiri sciureus* (2)	13	B	–	–
		312	B	–	–
Carnivores	*Helarctos malayanus* (4)	39	–	A	A
		40	–	A	–
		42	A	A	A
		46	A	A	A
Herbivores	*Oryx* (1)	45	–	A	–
	Damaliscus dorcas (1)	47	A	A	–
Birds	*Dromaius novaehollandia* (1)	16	B	B	B
	Acridotheres cristatellus (1)	67	B	–	–
Others	*Myrmecophaga tridactyla* (1)	306	B	–	–

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Table 3. Occurrence and assemblages of *G. duodenalis* for NHPs from different locations in China

Host location	Province/city	No. tested	No. (%) of positive specimens	Assemblage	Reference
Beijing Zoo	Beijing	12	0 (0)	–	Zhang *et al.* (2020)
Chengdu Zoo	Sichuan Province	47	6 (12.8%)	B	Zhang *et al.* (2020)
Changsha Zoo	Hunan Province	43	2 (4.7%)	B	Zhang *et al.* (2020)
Chongqing Zoo	Chongqing Province	33	0	–	Zhang *et al.* (2020)
Dalian Zoo	Liaoning Province	24	4 (16.7%)	B	Zhang *et al.* (2020)
Guiyang Forest Wildlife Zoo	Guizhou Province	58	7 (12.1%)	B	Zhang *et al.* (2020)
Guangzhou Zoo	Guangdong Province	8	0 (0)	–	Zhang *et al.* (2020)
Kunming Zoo	Yunnan Province	16	0 (0)	–	Zhang *et al.* (2020)
Nanjing Zoo	Jiangsu Province	16	0 (0)	–	Zhang *et al.* (2020)
Shaanxi Rare and Wildlife Zoo	Shaanxi Province	26	0 (0)	–	Zhang *et al.* (2020)
Suzhou Zoo	Jiangsu Province	10	4 (40.0%)	B	Zhang *et al.* (2020)
Yangzhou Zoo	Jiangsu Province	9	2 (22.2%)	B	Zhang *et al.* (2020)
Guiyang Zoo	Guizhou Province	50	15 (30%)	B	Zhong *et al.* (2017)
National experimental Macaque Reproduce Laboratory	Southwest China	31	0 (0)	–	Zhong *et al.* (2017)
Bifengxia zoo	Sichuan Province	24	0 (0)	–	Zhong *et al.* (2017)
Chengdu Gaoxin rhesus macaque farm	Sichuan Province	60	1 (1.7)	B	Zhong *et al.* (2017)
Chengdu zoo	Sichuan Province	9	0 (0)	–	Zhong *et al.* (2017)
Ya'an rhesus macaque base	Sichuan Province	30	0 (0)	–	Zhong *et al.* (2017)
Beijing Zoo	Beijing	72	16 (22.2)	A, B	Karim *et al.* (2015)
Wuhan Zoo	Hubei Province	66	5 (7.6)	B	Karim *et al.* (2015)
Taiyuan Zoo	Shanxi Province	66	9 (13.6)	B	Karim *et al.* (2015)
Changsha Wild Animal Zoo	Hunan Province	75	33 (44.0)	A, B	Karim *et al.* (2015)
Shijiazhuang	Hebei Province	89	10 (11.2)	B	Karim *et al.* (2015)
Shanghai Zoo	Shanghai Province	61	5 (8.2)	A, B	Karim *et al.* (2015)
Shanghai Wild Animal Zoo	Shanghai	67	14 (20.9)	B	Karim *et al.* (2015)
Qinling Mountains	Northwestern China	197	4 (2)	E	Du *et al.* (2015)
Commercial animal facility	Guangxi Province	205	5 (2.4%)	A, B	Ye *et al.* (2014)
Monkey farm and Zoo	Henan Province, Guangxi Province and Guangdong Province	1386	30 (2.2%)	B	Karim *et al.* (2014)
Qianling Park,	Guizhou Province	411	35 (8.5%)	A, B	Ye *et al.* (2012)
Shanghai Wild Animal Zoo	Shanghai	190	33 (17.4)	A, B	The current study

Molecular genotyping at the tpi *gene*

Based on analysis targeting *tpi* gene, assemblages A and B were found in 6 (20%) and 24 (80%) specimens, respectively, with assemblage B also being the major genotype. The sequence alignment indicated that six isolates (SHWD12, SHWD25, SHWD363, SHWD 365, SHWD366 and SHWD160) were identical to the human-derived isolates (KT948104). Five new assemblage subtypes were identified and the single-nucleotide polymorphisms (SNPs) are shown in the Supplementary Materials (Table S2). Using MF095053 as a reference sequence, isolate MT811043 has two SNPs at nucleotide positions 175 (A→G) and 522 (T→G); isolate MT811045 has two SNPs at 77 (T→C) and 175 (A→G); isolate MT811041 has two SNPs at 153 (C→T) and 522 (T→G); isolate MT811045 has one SNP at 250 (G→A) and isolate MT811045 has two SNPs at 250 (G→A) and 382 (G→A).

Discussion

Giardia duodenalis is one of the most common intestinal parasites resulting in waterborne and foodborne diarrhoea; it is also an important cause of traveller's diarrhoea. With the increasing reports on this parasitic outbreak, more and more investigations on this parasite in captive animals have been reported worldwide. The aim of this study was to investigate the prevalence and the zoonotic potential of *Giardia* based on genotypes by amplifying different loci in various captive animal species in a zoo in Shanghai, China.

In this study, the overall infection rate of *G. duodenalis* was 42 (6.2%) in 678 fecal samples examined from captive animals in zoological gardens. Zoonotic assemblages A ($n = 6$) and B ($n = 36$) were found, with assemblage B being more prevalent and in agreement with a previous study (Zhong *et al.*, 2017). In these animals involved in this study, the infection rate of NHPs was the highest (17.4%, 33/190). *Giardia duodenalis* infections in NHPs have been reported globally and the overall infection rate varied from 0 to 40% of examined *G. duodenalis* infection in NHPs in parks, zoos, farms or laboratories in China.

In the current study, the infection rate of NHPs was 17.4% and close to the prevalence in Dalian zoo (16.7%) (Zhang *et al.*, 2020). It was higher than those reported in Qianling Park in Guiyang

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(8.5%) (Ye *et al.*, 2012), Sichuan and Guizhou provinces (7.7%) (Zhong *et al.*, 2017), Qinling Mountain (2.0%) (Du *et al.*, 2015) and two other additional comprehensive parasite infection studies in China (2.2 and 1.3%) (Ye *et al.*, 2012; Li *et al.*, 2017) Table 3. We can find that different areas have different *G. duodenalis* infection rates in NHPs in China due to a range of factors, including the diagnostic method and study design, geographical conditions, number of samples and sampling season. Our results and those of previous studies indicate that *G. duodenalis* infection is common in NHPs and has a wide geographic distribution in China.

In other countries in the world, the overall infection rate of *G. duodenalis* in NHPs is between 2.2 and 47.0% (Berrilli *et al.*, 2011; Karim *et al.*, 2014). Reports of the prevalence rate of giardiasis in NHPs from different countries varies from 6.0% in Italy (Berrilli *et al.*, 2011) and 7.0% in Thailand (Sricharern *et al.*, 2016), and it was lower than 11.1% in Uganda (Johnston *et al.*, 2010) and 50% in Croatia (Beck *et al.*, 2011). The different infection rates may be explained by diagnostic methods, the climate among these regions, environmental management and NHP species. We also observed that the high infection rate of NHPs could be attributed to the frequent contact within the range of activities. It can actually increase the risk of *G. duodenalis* transmission.

Based on MLG, the MLG model was used to better understand the infection and genetic characteristics of *G. duodenalis* in animals and humans, which is helpful for unveiling zoonotic potential and dynamic transmission. In our study, the genetic diversity of these positive *G. duodenalis* isolates was determined by the amplification and sequencing of the *gdh*, *tpi* and *bg* genes, with 30 *gdh*, 30 *tpi* and 22 *bg* gene sequences being obtained (Table 2). *gdh* and *tpi* genes gave a higher PCR amplification rate. Molecular characterization of *G. duodenalis* isolates revealed that all the infected NHPs were zoonotic assemblage B, which is consistent with previous reports (Johnston *et al.*, 2010; Ryan *et al.*, 2013; Karim *et al.*, 2015). At *bg* locus, one isolate (SHWD185) and three isolates (SHWD159, SHWD168 and SHWD357) were identical to the human-derived isolates (MG746614 and MG736242). At *gdh* locus, one isolate (SHWD367) showed complete homologous to the reference sequence of the human-derived isolate (MH311013). Six isolates have 100% similarity with human-derived isolates (GenBank no. KT948104) based on the *tpi* gene (Wegayehu *et al.*, 2016). The sequence data indicated these animals could be potentially infectious to other animals or surroundings, and further study is essential to clarify the transmission. In addition, we also observed a high genetic diversity in assemblage B subtypes at *tpi* locus.

Giardia duodenalis assemblages A and B are zoonotic and can infect a broad range of hosts including humans. However, fecal–oral route is the primary way of *G. duodenalis* transmission. Ingestion of infective cysts through contaminated water and food is the common transmission route. To the best of our knowledge, during the past decade, more and more studies have showed that the close correlation between human activity and the occurrence of wildlife diseases (Thompson *et al.*, 2010; Brearley *et al.*, 2013). In this zoo, different kinds of animals such as tigers, lions or birds lived in a relatively independent environment. However, they could be indirectly in contact with humans through the surrounding water. In addition, breeders and visitors have great chance to get in touch with some kinds of caged birds and NHPs. Previous studies have reported that the most *Giardia* infections naturally occurring in wildlife may be related to the wildlife habitats, and influenced by direct contact or environmental routes (Thompson, 2013; Abeywardena *et al.*, 2015). In fact, cross-species transmission among the same kinds of animals is possible, and cleaning up the feces in time can reduce the pollution to a great extent. However, different kinds of animals have little chance to be in contact with each other because they lived in a relatively independent environment. Therefore, the feeding and management strategies should be improved and the disease surveillance should also be strengthened to prevent *Giardia* infection. For example, we suggest that zoo manager can reduce the frequency of human–animal contact, increase the construction of zoo zoning and clean up the feces excreted by animals in time to reduce the pollution to the surrounding environment, animals or humans. In the future studies, the breeders and water sources around these animals, in addition to the other animals in contact with them should also be investigated for the occurrence of *G. duodenalis* and its genetic characteristics. Also, the effect of *Giardia* infection on the health of captive animals in zoological gardens needs to be resolved.

Conclusions

Our study revealed the presence of *G. duodenalis* in captive animals in zoological gardens and new isolates were identified in NHPs. Assemblages A and B were found with assemblage B being dominated. The sequences were 100% homologous with registered human-derived sequences in the GenBank indicating the potential possibility of zoonotic transmission. Nevertheless, to evaluate whether the surrounding environmental contamination caused by these animals or other anthropogenic activities, the detection of more specimens including water, other animals or human feces will be involved in further study.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182021000913

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Author contributions. YS, HL and JC conceived and designed the study. BW and ZY conducted sample collection. BW and HL performed laboratory experiments. YJ, JZ and JY performed statistical analyses. HL wrote the manuscript. YS revised the manuscript. All authors have read and approved the final version of the manuscript.

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Conflict of interest. None.

Ethical standards. Prior to collection of fecal specimens, permission was obtained from zoo managers. No specific permits were required for the described field studies. All fecal specimens were collected from the ground using plastic bags the following day. Animals were not harmed in any way during the procedure. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention. The protocol was approved by the Laboratory Animal Welfare & Ethics Committee (LAWEC), National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (No. 2014001).

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