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SHORT COMMUNICATION

First report of *Giardia lamblia* in different animals in the United Arab Emirates

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ABSTRACT

The aim of this study was to detect and characterize *Giardia lamblia* in animals in the UAE. Eighty-seven fecal samples were tested for *G. lamblia* using the conserved fragment of small subunit (SSU)-rRNA by nested PCR. *Giardia*-positive isolates were genotyped for assemblages A and B using assemblage specific primers of the triosephosphate isomerase (*tpi)* gene. Thirty samples (34.5%) were positive for *G. lamblia*. Conversely, neither genotype A nor B were detected using *tpi* genotyping on the studied samples. Further investigations are required using higher number of samples including both human and animals in the country taking into consideration the analysis of other genotypes to provide more detailed understanding about the zoonotic transmission of this parasite.

Keywords: *Giardia lamblia*; SSU-rRNA gene; PCR; Animals; United Arab Emirates.

INTRODUCTION

Giardia lamblia is one of the most important zoonotic enteric parasites (Feng & Xiao, 2011; Zhang *et al.,* 2016). To date, eight assemblages (A-H) have been identified (Ramirez *et al.,* 2015; Santin & Fayer, 2015; Zhang *et al.,* 2016). While assemblages C-H appear to be animal host specific, assemblages A and B are potentially zoonotic infecting both humans and a diverse range of non-human hosts (Ryan & Cacciò, 2013; Mohamed *et al.,* 2014; Zhang *et al.,* 2016). Although *G. lamblia* infections have been reported in humans and a variety of animal species (Cacciò & Ryan, 2008; Bouzid *et al.,* 2015; Ehsan *et al.,* 2015; Li *et al.,* 2015), little or no information is available on the prevalence of *G. lamblia* and the various assemblages among animals in UAE. Given the understanding that additional epidemiological data is needed to identify potential animal reservoirs of human cases of *G. lamblia* infections, the primary objective of the present study was to detect and genotype *G. lamblia* in different animal groups in the UAE.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

The samples used in this study were part of another survey published earlier (AbuOdeh *et al.,* 2019). Briefly, 87 fresh fecal samples were obtained from various animal species across the UAE and stored at –20°C until submitted to DNA extraction and PCR (Table 1). Care was taken to reduce the risk of sample contamination. DNA was extracted from all stool samples using the Bioline stool DNA Kit (London, UK) as per the manufacturer's recommendations and stored at –20°C until analyzed.

PCR amplification and DNA Sequencing

Conserved fragment from the small subunit (SSU)-rRNA gene was amplified by nested PCR using the outer primers RH11 and RH4 as previously described (Hopkins *et al.*, 1997), and the nested primer pair GiarF and GiarR (Read *et al.,* 2002). The primers' sequences were: RH11 (5'CAT CCG GTC GAT CCT GCC3'), RH4 (5'AGT CGA ACC CTG ATT CTC CGC CAG G3') (Hopkins *et al.*, 1997) and GiarF (5'GAC GCT CTC CCC AAG GAC 3'), GiarR (5' CTG CGT CAC GCT GCT CG 3') (Read *et al.*, 2002), amplifying 130 bp fragment. The reaction mixture for PCR contained 5 µL of 5X PCR reaction buffer, 13 µL of nuclease free water, 0.5 µL of dNTP, 0.5 µL of each primer (RH4 and RH11), 0.5 µL of Taq DNA polymerase and 5 µL of the DNA template. For nested PCR, 1 µL of the amplified sample was used as a template instead. A negative control was included to rule out false-positive. The PCR reactions was performed in Eppendorf Master Cycler Thermocycler (Eppendorf; Hamburg, Germany) according to the following conditions: initial denaturing step at 96°C for 4 minutes, followed by 30 cycles, each comprising denaturation at 96°C for 20 seconds, an annealing step at 59°C for 20 seconds and extension at 72°C for 30 seconds at 4°C until further processing. PCR products were sent for sequencing in a commercial sequencing facility (Molecular Cloning Laboratories; San Francisco, CA, USA). The SSU rRNA gene sequences obtained in this study were deposited in GenBank under accession numbers MT129471-MT129497.

For detection of genotypes A and B, nested PCR was done on all SSU-rRNA-positive samples to amplify the variable locus triose phosphate isomerase (*tpi*) gene using the primers designed by Amar and colleagues (2003) with slight modifications. The reaction mixture (25 µl in total) consisted of 5 µL of 5X PCR buffer, 13 µL of nuclease free water, 0.5 µL of dNTP, 0.5 µL of each primer, 0.5 µL of Taq DNA Polymerase and

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Table 1. Animal sources and *G. lamblia* detection percentages in the various animal groups

Host	Scientific Name	Number of Samples	*G. lamblia* Positive Samples No. (%)	Assemblage*		
				A	B	Other(s)
Artiodactyla			9 (40.9%)	0	0	ND
Cow	*Bos Taurus*	11				
Sheep	*Ovis aries*	5				
Goat	*Capra hircus*	6				
Aves			6 (33.3%)	0	0	ND
Domestic duck	*Anas platyrhynchos domesticus*	2				
Quail	*Coturnix coturnix*	4				
Peacock	*Pavo cristatus*	1				
Pigeon	*Oena capensis*	4				
Parrot	*Psittacula krameri*	3				
Chicken	*Gallus gallus*	4				
Carnivora			1 (33.3%)	0	0	ND
Domestic cat	*Felius catus*	2				
Mongolian ferret	*Mustela putorius furo*	1				
Lagomorpha			2 (100%)	0	0	ND
Rabbit	*Oryctolagus* spp.	2				
Perciformes			5 (41.6%)	0	0	ND
Fish	*Oreochromis niloticus*	12				
Rodentia			7 (78%)	0	0	ND
Squirrel	*Funambulus palmarum*	5				
Mongolian Chinchilla	*Chinchilla lanigera*	1				
Shrew-faced squirrel	*Rhinosciurus laticaudatus*	1				
White Squirrel	*Sciurus carolinensis*	1				
Hamster	*Mesocricetus auratus*	1				
Squamata						
Burmese snake	*Python bivittatus*	1				
Rainbow Snake	*Farancia erytrogramma*	2				
Boa Salmon Snake	*Boa constrictor*	1				
Granite Albino python South East Asia	*Python bivittatus*	1				
Albino Burmese Python Southern Asia	*Python bivittatus*	1				
Iguana from Southern Asia	*Iguana iguana*	1				
Chameleon from India	*Chamaeleo zeylanicus*	1				
Bearded Dragon from the Sahara Desert	*Pogona vitticeps*	1				
South Asian Gecko	*Hemidactylus frenatus*	2				
Testudinidae						
African spurred tortoises	*Centrochelys sulcate*	7				
Syrian tortoise	*Testudo graeca*	1				
Mata Mata turtle from South America	*Chelus fimbriata*	2				

– ND: Not Done.
– * See Discussion (line 90).

5.0 µL of the DNA template was added to the mix. After an initial denaturation step at 94°C for 4 minutes, 30 cycles with denaturation at 94°C for 30 seconds, annealing at 52°C for 30 sec, 72°C for 1 minute and a final extension at 72°C for 10 min were performed. For the nested PCR 1 µL of the initial PCR product was used as template DNA. The conditions for the nested PCR were identical to the primary PCR except for the annealing temperature which was raised from 52°C to 60°C for genotype A and 54°C for genotype B. In addition, the cycle number was raised to 33 cycles. The amplified products were analyzed using gel electrophoresis in a 1.8% agarose gel.

RESULTS AND DISCUSSION

Of the 87 samples tested, 30 (34.5%) were confirmed by SSU rRNA sequencing as positive for *G. lamblia*. The following infection rates were detected in animal groups: rodentia, 7/9 (78%) [*Chinchilla lanigera* – 1; *Funambulus palmarum* – 5; *Mesocricetus auratus* – 1]; aves, 6/18 (33.3%) [*Psittacula krameria* – 1; *Coturnix coturnix* – 2; *Oena capensis* – 1; *Gallus gallus* – 2]; perciformes, 5/12 (41.6%) [*Oreochromis niloticus* – 5]; carnivora, 1/3 (33.3%) [*Felius catus* – 1]; lagomorpha, 2/2 (100%) [*Oryctolagus* spp. – 2]; artiodactyla, 9/22 (40.9%) [*Bos taurus* – 3; *Ovis aries* – 1; *Capra hircus* – 5]. All 30 samples were further assayed for the *tpi* A and B genes to determine those assemblage(s) distribution. Neither assemblage A nor B were detected among the samples.

To our knowledge, no animal studies on the detection and genotyping of *G. lamblia* infections has been done in the UAE. These current findings confirm the presence of *G. lamblia* in animals. Furthermore, the absence of potential zoonotic genotypes (assemblages A and B) in the present report does not contradict its presence among the studied animal groups. Thus, more samples should be assayed to determine their presence including animals from other groups. Many studies have reported various infection rates in different animal

families (Feng & Xiao, 2011). Interestingly, the present study is the first of its kind to detect *G. lamblia* in *Oreochromis niloticus* fish (41.6%). A similar study in Egypt reported an overall infection rate of 3.3% in this fish collected from fish farms and the Nile River, with a detection rate of 2.9% in genotype A, being the only assemblage detected (Ghoneim *et al.,* 2012). However, a more detailed study about the zoonotic transmission of this parasite should include more samples as well as the analysis of other genotypes (C, D) and livestock genotype (E). In studies of *G. lamblia* isolated from cattle, genotype E was detected with a prevalence between 56 and 57% (O' Handley *et al.,* 2000). This genotype has no zoonotic risk (Matsubayashi *et al.,* 2005). However, in a recent study in Brazil, assemblage E was detected in humans suggesting a new zoonotic route of transmission, although we did not test for this assemblage in the current study (Fantinatti *et al.,* 2016). It is too soon to underline the possibility of any of the animal groups studied as reservoirs of zoonotic *G. lamblia* assemblages. Genotypic studies carried out with more human and animal samples from different areas will increase our understanding of the epidemiology of giardiasis in the UAE.

Conflict of interest

Authors declare no conflict of interest of any sort with anyone.

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