

Prevalence and genotype of *Giardia duodenalis* from faecal samples of stray dogs in Hualien city of eastern Taiwan

Tseng, Y.C.¹, Ho, G.D.², Chen, T.T.W.³, Huang, B.F.⁴, Cheng, P.C.⁵, Chen, J.L.⁶ and Peng, S.Y.^{7*}

¹Institute of Medicine and Biotechnology, College of Medicine, Tzu Chi University, Hualien, Taiwan

²Effpha Pharma Management Corp. Taipei Taiwan

³Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan

⁴Department of laboratory medicine, Buddhist Tzu-Chi General Hospital, Hualien, Taiwan

⁵Department of Parasitology and Center for International Tropical medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

⁶Department of Medical Informatics, College of Medicine, Tzu Chi University, Hualien, Taiwan

⁷Department of Biochemistry, College of Medicine, Tzu Chi University, Hualien, Taiwan

*Corresponding author email: pengsy@mail.tcu.edu.tw

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Abstract. *Giardia duodenalis* is a zoonotic protozoan parasite that causes diarrhea through waterborne transmission or fecal-oral infection. The cysts are chlorine-resistant and, therefore, can pollute drinking water and induce a pandemic disease. In this study, we aimed to detect *G. duodenalis* infection in stray dogs in Hualien, Taiwan. We collected faecal samples from 118 dogs and amplified DNA sequences of the β -*giardin* gene by nested polymerase chain reactions (nested PCR). Eleven of the 118 faecal samples tested positive for the parasite. The genotype analysis of the 11 samples indicated that 7 samples belonged to assemblage C and four samples belonged to assemblage D. Our study provided a better understanding of the infection rate and genotypes of *G. duodenalis* in dogs from Hualien City, and human infection could not be induced by this zoonotic infection pathway in Hualien City.

INTRODUCTION

Giardia duodenalis (also known as *G. lamblia* and *G. intestinalis*) is a flagellated eukaryotic unicellular protozoan that is known to cause diarrhoeal diseases worldwide. There are two stages in its life cycle: cyst and trophozoite. Infection by *G. duodenalis* occurs through ingestion of cysts and results in the symptoms of diarrhea and malabsorption. This protozoan has been observed in humans with 200 million symptomatic giardiasis each year (World Health Organisation, 1996) and in other mammals (Thompson, 2000).

Giardia duodenalis consists of a number of genotypes or assemblages, specifically A–H (Thompson & Monis, 2004; Monis *et al.*, 2009; Feng & Xiao, 2011). Assemblages A and

B have been found to comprise anthropophilic pathogens (Lalle *et al.*, 2005b), although several animal species, including dogs and cats, have been found to be infected with both assemblages (Thompson, 2004; Xiao & Fayer, 2008). Assemblages C, D, F, and G mainly infect non-human species (Thompson *et al.*, 2008; Monis *et al.*, 2009), and assemblage H has been identified only in marine vertebrates (Lasek-Nesselquist *et al.*, 2010). Several papers, however, have confirmed that dogs can carry both the zoonotic (assemblage A) and the canine-specific (assemblages C and D) subtypes of *G. duodenalis* (Wielinga & Thompson, 2007; Eligio-Garcia *et al.*, 2008; Papini *et al.*, 2009).

Previously, a single publication by Liang *et al.* (2012) reported *G. duodenalis* infection in dogs in Nantou County, Taiwan. Our goal is

to expand our knowledge about the infection and genotypes of *G. duodenalis* in a separate location, Hualien City, Taiwan.

MATERIALS AND METHODS

Sample collection

The eastern Taiwanese city of Hualien, with a humid subtropical climate, mean annual temperature of 23.3°C, average relative humidity of 78%, and average annual rainfall of 2157 mm, has favorable environmental conditions to support the spread of parasitic diseases. Coupled with more than 2000 stray dogs living in Hualien (http://animal.coa.gov.tw/html/?main=9h&page=09_resources_a02), this leads to more than just a nuisance but a significant potential source of infectious disease.

We collected 118 fresh, stray dog faecal samples from streets or shelters in Hualien City, from July 2010 to June 2011. In order to avoid double sampling from an individual, we collected stool samples at different times and places. Each faecal sample (approximately 2–5 g) was collected with a wooden tongue depressor. Samples were transported to our laboratory in iceboxes maintained at 4°C and were stored at 4°C for future processing.

Parasite examination by microscopy

The stool consistency of all the samples (n = 118) was recorded. The fresh smear was prepared using a small portion (100 mg) of a faecal sample and a drop of Lugol's iodine solution for light microscopy analysis to check for *Giardia* cysts and trophozoites (Cho *et al.*, 1990). The samples were considered as positive if at least one *G. duodenalis* cyst or trophozoite was detected.

DNA analysis

Genomic DNA was extracted from each *Giardia*-positive faecal sample by using an UltraClean® Fecal DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA, USA) according to the manufacturer's instructions, and the extracted DNA was stored at -20°C.

In the first PCR reaction, we amplified a 753-basepair (bp) fragment of the β -*giardin* gene by nested PCR (Lalle *et al.*, 2005a, b), using the forward primer (G7: 52-AAGCCCGACG ACCTCACCCG CAGTGC-32 and reverse primer G759: 52-GAGGCCGCCCTGGATC TTCGAGACGAC-32 (Cacció *et al.*, 2002). In the second step of the PCR (sequential reaction of nested PCR), a 511-bp fragment was amplified by the forward primer (G99: 52-GAACGAGATCGAGGTCCG-32 and reverse primer (G609: 52-CTCGACGAGC TTCGTGTT-32) (Lalle *et al.*, 2005b). The β -*giardin* positive control used in this study was the reference strain of assemblage A1 (WB clone C6; strain ATCC 50803; GenBank accession number M36728).

PCR amplification

A total reaction volume of 50 μ L consisting of 2.5 μ L genomic DNA (acquired from dog faeces), 1x PCR buffer containing 1.5 mM MgCl₂, 0.5 μ M of each primer, 200 μ M of each dNTP, and 1 unit of GeNei Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India) was used. In the sequential PCR, 2.5 μ L of the amplified DNA from the first reaction was used as the template, and the subsequent PCR was performed using a GeneAmp PCR 2700 system (Life Technologies). Amplifications by PCR was performed for 40 cycles under the following conditions: denaturation at 94°C for 30 sec, annealing at TM°C for 30 sec (first PCR: 60°C, second PCR: 55°C), extension at 72°C for 40 sec, and a final extension at 72°C for 7 min.

DNA sequence analysis

The amplified PCR products and appropriate sequencing primers were sent for purification and sequencing to Tri-I Biotech, Inc., Taiwan. The sequences were checked and compared using BioEdit software (Hitachi Software Engineering, Tokyo, Japan; <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). The GenBank accession numbers for the sequences (GD1, GD2, GD3, GD4, GD5, GD-6, and GD-7) are JX867765–JX887771, respectively.

RESULTS

We detected a single parasite species, *G. duodenalis*, in 11 dog samples (9.3% of the total samples) by microscopic examination and genetic analysis. The sequence analysis revealed that seven positive samples (5.93%, sample No. D1, 8, 15, 36, 38, 74, and 90) belonged to assemblage C (accession number AY545646), and the other four positive samples (3.39%, sample No. D9, 98, 105, and 117) belonged to assemblage D (accession number AY545648). From the samples that belonged to assemblage C, two samples were 100% identifiable with assemblage C (accession number AY545646), and the other five samples could be divided into five isolates (GD-1, GD-2, GD-3, GD-4, and GD-5), which differed from assemblage C by 1–3 nucleotides (Figure 2A, Tables 1 and 2). From the samples that belonged to assemblage D, two samples were 100% identifiable with assemblage D (accession number AY545648), and the other

two samples could be divided into two isolates (GD-6 and GD-7) that differed by 1–3 nucleotides from assemblage D (accession number AY545647; Figure 2B, Tables 1 and 2).

DISCUSSION

This paper is the first to report *G. duodenalis* infection in stray dogs in Hualien, Taiwan. We predicted that these dogs could cause zoonosis transmitted infections by *Giardia*. To test our prediction, we used microscopic and genetic analyses to detect *G. duodenalis* infection and to identify its assemblage.

There is little information available regarding the current levels of infection in dogs in Taiwan. Only Liang *et al.* (2012) reported the rate of *Giardia* infection in dogs in Nantou County, Taiwan, at 9.52% (4/42) in 2012. Our study showed a similar prevalence of 9.3% (11/118) in Hualien. However, the major infectious genotype of *G. duodenalis*

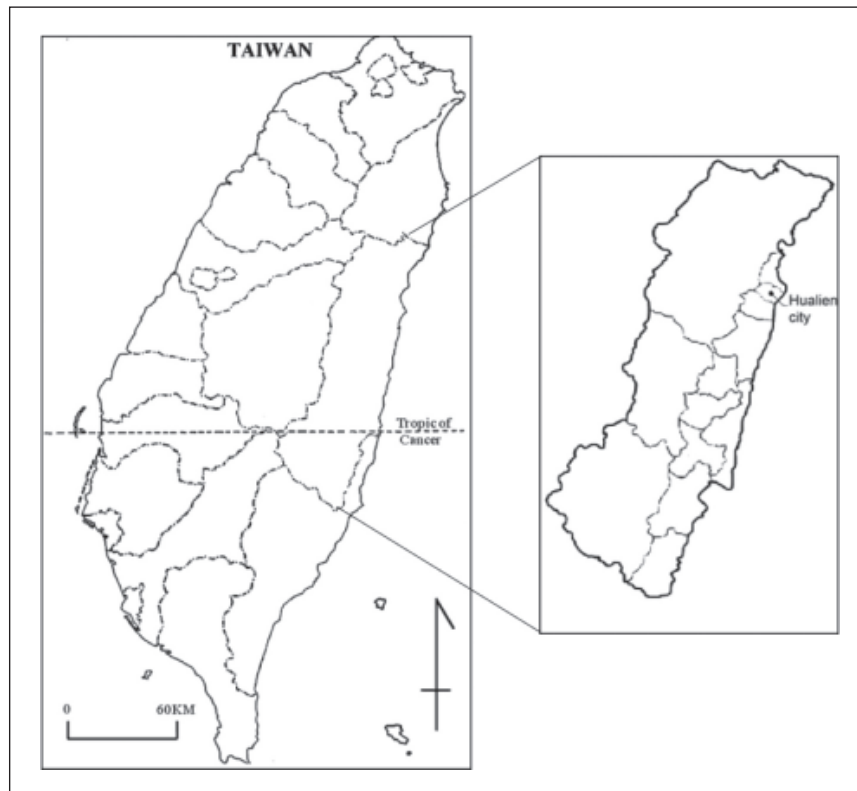


Figure 1. The map indicates Hualien city in Hualien County of Eastern Taiwan

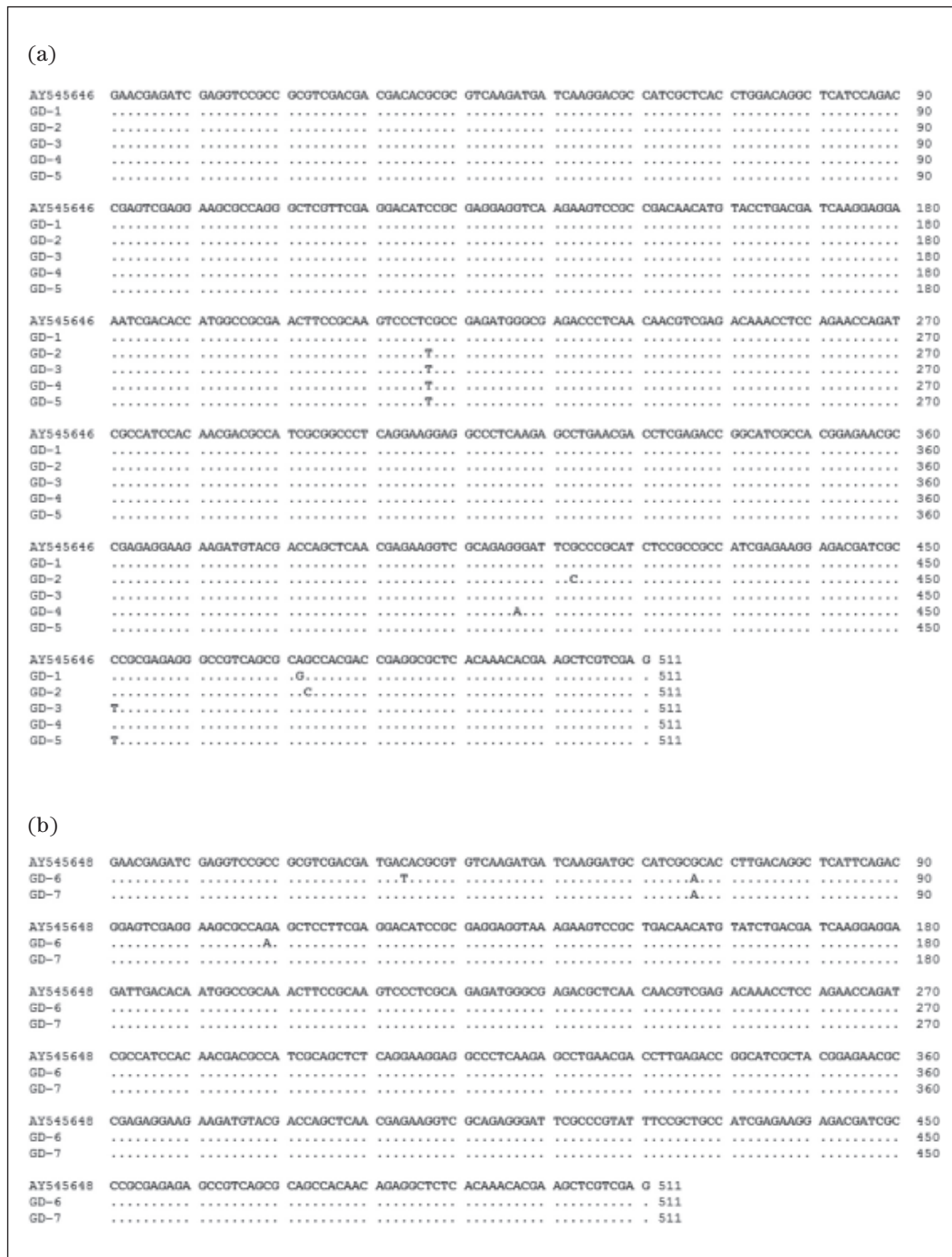


Figure 2. The nucleotide sequences of the *G. duodenalis* β -giardin gene. Among 11 positive samples obtained from dogs, the sequences from 2 samples had 100% homology with assemblage C (accession number AY545646) and 2 samples 100% homology with assemblage D (accession number AY545648), and the other 7 were divided into 7 isolated groups (GD-1 to 7). GD-1~5 were belong to assemblage C (Fig. 2A), and GD-6 and GD-7 were identified as belonging to assemblage D (Fig. 2B)

Table 1. PCR result of the investigation of the β -giardin gene

No. of positive samples	Assemblage by sequencing analysis	Submit GenBank Accession No.
D8	C	JX867765 (GD1)
D15	C	JX867766 (GD2)
D36	C	JX867767 (GD3)
D38	C	JX867768 (GD4)
D74	C	JX867769 (GD5)
D1, D90	C	AY545646*
D98	D	JX867770 (GD6)
D117	D	JX867771 (GD7)
D9, D105	D	AY545648*

* The gene sequence is 100% identity with AY545646 or AY545648

Table 2. The sequences of 7 dog samples (GD-1, GD-2, GD-3, GD-4, GD-5, GD-6 and GD-7) compared to assemblage C (AY545646) or assemblage D (AY545648)

Sequence	Position								
	34	67	109	216	407	413	451	472	473
assemblage C									
AY545646	-	-	-	C	G	G	C	A	G
GD-1	-	-	-	-	-	-	-	G	-
GD-2	-	-	-	T	-	C	-	-	C
GD-3	-	-	-	T	-	-	T	-	-
GD-4	-	-	-	T	A	-	-	-	-
GD-5	-	-	-	T	-	-	T	-	-
assemblage D									
AY545648	C	G	G	-	-	-	-	-	-
GD-6	T	A	A	-	-	-	-	-	-
GD-7	-	A	-	-	-	-	-	-	-

was different between Nantou (75% assemblage D, 3/4) and Hualien (64% assemblage C, 7/11). As the Central Mountain Range separates these two locations, the genetic differences may not be surprising, and we considered the *Giardia* infection in Nantou and Hualien to be unrelated. *Giardia* infection seems to be common in Taiwan as it has been reported in children from several locations including Taipei County (3.8% in 1976), Nantou County (7.2% in 1979 and 3.83% in 2012), Orchid Island (10.2% in 2001), and Kaohsiung County (2% in 2000) (Yu & Chiu,

1976; Chiu *et al.*, 1979; Lee *et al.*, 2000; Tsaihong *et al.*, 2001; Liang *et al.*, 2012).

Other studies reported a similar *Giardia* prevalence of 9.3% in Australia (Palmer *et al.*, 2008), 7.2% in Canada (Jacobs *et al.*, 2001), and 8.1% in Alberta, Canada (Joffe *et al.*, 2011). Although studies have shown that dogs can carry assemblage A, C, and D subtypes of *G. duodenalis*, our study revealed that the dogs in Hualien were infected by *Giardia* assemblages C and D, whereas assemblages A and B have yet to be detected.

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