

Does Silent *Giardia* Infection Need Any Attention?

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Abstract: Even though treatment of symptomatic cases as well as improving hygiene and sanitation conditions decrease giardiasis in underdeveloped and/or developing countries, *Giardia duodenalis* infection is still frequently encountered especially in children living in crowded conditions leading to public health problems. We studied the prevalence of *G. duodenalis* infection in 274 asymptomatic Thai boy orphans, living in/around the Bangkok metropolitan area, by use of direct fecal smear and formalin-ethyl acetate techniques. This was done to explore our hypothesis that children with asymptomatic giardiasis, who are not likely to be treated, have a high potential for spreading this pathogen. Positive *Giardia* cases were further genotyped by 2 genes: small subunit ribosomal RNA (*ssrRNA*) and glutamate dehydrogenase (*gdh*) to determine the distribution of each *G. duodenalis* assemblage. Positive *G. duodenalis* infection was 11.67 % and revealed at 10.63%, 12% and 15%, in PK, TMK, and MHK orphanages, respectively. The overall parasitic infection, including *Giardia*, was 48.54% consisting mostly of non-pathogenic organisms i.e. *Blastocystis hominis*, *Entamoeba coli*, *Endolimax nana*, *Entamoeba histolytica*-like, *Chilomastix mesnili*, *Trichuris trichiura*, *Hymenolepis nana*, *Strongyloides stercoralis* and Hookworm. Fourteen (70 %) and 4 (20 %) out of the 20 positive *Giardia* samples could be amplified by *ssrRNA* and *gdh* genes, respectively; three belonged to the sub-assemblage BIV and only one indicated sub-assemblage AII. Thus children infected with sub-assemblage BIV isolate are more common in this study. In conclusion, we focused on asymptomatic giardiasis children, hitherto unrecognized, who consistently contaminate their environments with cysts. We favor not only treating these children to eradicate the source of the infection, but also to encourage an active surveillance program consisting of highly sensitive methods to identify silent giardiasis cases. In addition to these 2 strategies, a continuous fruitful health education program for all stakeholders is another crucial tool. These actions will benefit the control of human giardiasis and reduce public health problems.

Keywords: *Giardia duodenalis*, asymptomatic Thai boy orphans, genotype, small subunit ribosomal RNA, glutamate dehydrogenase.

INTRODUCTION

Giardia duodenalis is the most frequent intestinal protozoa found throughout the world [1]. Most infections result from fecal-oral transmission by ingestion of contaminated water and food which are the major vehicles for transmitting the infection [2,3]. *G. duodenalis* is clearly associated with children living in crowded conditions, such as in childcare centers or orphanages. Moreover, this protozoan eventually produces intermittent and chronic gastrointestinal symptoms leading to public health problems [4,5]. Children infected with *G. duodenalis* are irritable and have behavioral problems. They fail to thrive and suffer growth failure because of malabsorption [6]. Asymptomatic infected children, who do not demonstrate any obvious clinical signs and symptoms, persistently contaminate their environment by shedding cysts and thus unknowingly transmitting *G. duodenalis* to new hosts.

In Thailand, giardiasis is still a significant problem in children. The rate of *G. duodenalis* infection depends on the

children's age, their level of personal hygiene, and local sanitary conditions [7]. Several studies show a continued high prevalence of giardiasis in Thailand [8-10]. Saksirisampant *et al* reported a high prevalence of human giardiasis in pre-school orphans as well as in pre-school children aged 3 months to 5 years in rural areas of Thailand [11,12]. Some children carry *G. duodenalis*, but they are asymptomatic. These carriers have a high potential for spreading this pathogen making the situation worse in at-risk areas.

G. duodenalis is comprised of at least 7 assemblages: A, B, C, D, E, F and G [13] but only assemblages A (AI, AII) and B (BIII, BIV) have been recovered from human [14]. Metabolism and growth rate may be the important factors in regulating phenotypic differences between assemblages A and B. It may be suggested that there are differences in the nature of infection between assemblages A and B, including duration of infection, drug sensitivity and virulence [15]. Amar and colleagues reported that children infected with assemblage A isolates had greater diarrheal symptoms than children infected with assemblage B isolates [16]. Children infected with assemblage B isolates easily spread *G. duodenalis* cysts in communities by unknowingly shedding them. In this study, we examined asymptomatic Thai boy orphans living in 3 orphanages located in/around Bangkok in order to investigate *G. duodenalis* and other intestinal

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parasitic infections. Genotyping *G. duodenalis* in positive stool samples was carried out to determine the distribution in each *G. duodenalis* assemblage. We believe that the obtained data can provide significant insight into *G. duodenalis* infection, and thus effective strategies for the prevention and treatment of giardiasis, especially in crowded conditions, will be administered.

MATERIALS AND METHODS

Study Subjects

Two hundred and seventy-four Thai boy orphans from 3 orphanages (PK, MHK and TMK), located in/around Bangkok, were enrolled in the study from August to December 2009. The subjects ranged in age from 5-15 years and were comprised of 94, 100 and 80 children from PK, MHK and TMK homes, respectively. All participants were asymptomatic and healthy at the time of the study.

Ethical Approval

The study protocol was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand (MUTM 2009-032-01). Written informed consents were obtained from all participants and from the guardians of THE children before stool samples were collected.

Stool Sample Collection

A verbal explanation and specific instructions for sampling and avoidable contamination of the stool samples were given to all the children and guardians. Fresh stool samples were collected in the early morning and transported in proper conditions to the laboratory for microscopic examination at the Department of Protozoology, Faculty of Tropical Medicine, Mahidol University.

All stool samples were examined by direct fecal smear and formalin-ethyl acetate techniques. The presence of intestinal parasites was investigated by two independent well-trained microscopists. Positive cysts, trophozoites, eggs or larvae were identified based on diagnostic criteria of shape, size, color and special characters of those intestinal parasites according to the Atlas of medical parasitology: with 465 colour illustrations [17]. All the positive stool samples for *G. duodenalis* were genotyped and sub-genotyped.

DNA Extraction and Genotyping

DNA was extracted from all the *G. duodenalis* positive samples using a Flexi Gene DNA kit (Qiagen, Hilden, Germany). Unfortunately, some *G. duodenalis* positive stool samples were too small to extract, therefore we excluded them. All together there were 20 stool samples for DNA extraction. DNA samples were stored at -20°C until further use.

Genomic DNA was amplified by PCR using the specific pairs of oligonucleotide primers. Twenty positive samples of *G. duodenalis* were genotyped by 2 genes: small subunit ribosomal RNA (*ssrRNA*) gene and glutamate dehydrogenase (*gdh*) gene. The *ssrRNA* gene provides screening in all assemblages of *G. duodenalis* infection, whilst the *gdh*

gene is useful for grouping them into their genetic assemblages and also into sub-assemblages AI, AII, BIII and BIV. The single-round 117- bp PCR product of *ssrRNA* gene was amplified by forward primer NewGiarF (5'-ACG CTC TCC CCA AGG ACG-3') and reverse primer NewGiarR (5'-GGT TGC CAG CGG TGT CC-3'). PCR reactions were performed on total volumes of 50 μL using 2.5 μL of genomic DNA template, 300 nM of each oligonucleotide primer, 200 μM of dNTPs (dATP, dCTP, dGTP, dTTP), PCR buffer 10mM Tris-HCl (pH 8.3), 50 mM of KCl, 1.5mM of MgCl_2 , 1.0 U of *Taq* polymerase. DNA amplification was performed in a Gene Amp PCR system 9700 programmed (Applied Biosystem, USA) and carried out at 95°C for 5 min, followed by 35 cycles in which each cycle took 30 seconds at 95°C denature, 30 seconds at 58°C annealing temperature, and 30 seconds at 72°C extension, and a final extension step of 72°C for 4 min. For the semi-nested PCR, the 432- bp PCR product of *gdh* gene was amplified by external forward primer GDHeF (5'-TCA ACG TAA AGC GAG GAT TCC GT-3'), internal forward primer GDHiF: (5'- CAG TAC ACC TCA GCT CTC GG-3') and reverse primer GDHiR (5'- GTT CTC CTT GCA CAT CTC C-3') [18], respectively.

Restriction Fragment Length Polymorphism (RFLP) Analysis

The 432 bp PCR product of *gdh* gene was digested with 10 U of *NlaIV* for 3 hours at 37°C or 5 U of *RsaI* for 3 hours at 37°C . The *NlaIV* restriction enzyme can digest 432 bp PCR products which restriction digest fragments can distinguish between sub-assemblages AI and AII, and assemblage B. Diagnostic genotyping profile (bp) of *G. duodenalis* among assemblages when digested by *NlaIV* was as follows: 90, 120, 150 (AI); 70, 80, 90, 120 (AII); 120, 290 (B), respectively. Additionally, *RsaI* digestion restriction enzyme distinguished between sub-assemblage BIII and BIV. Diagnostic genotyping profile (bp) of *G. duodenalis* distinguished between assemblage A, sub-assemblages BIII and BIV was as follows: 280, 130 (A); 130, 300 (BIII); 430 (BIV), respectively. The restriction digest fragments were separated on 3.0% agarose gel, stained with ethidium bromide to visualize the different genotypes, and measured for fragment size by reference to a 50 bp DNA ladder marker. Moreover, the 432 bp PCR products of *gdh* gene were sequenced to confirm RFLP analysis as well.

Statistical Analysis

Descriptive analysis was used with percentages to express the prevalence of *G. duodenalis* and other intestinal parasitic infections.

RESULTS

The overall prevalence of intestinal parasitic infection in 274 Thai boy orphans was 133 (48.54%), and there were 34 cases of *Giardia* infection (12.41 %). The highest prevalence was found at MHK home with an infection rate of 53.75 % compared with 46.73% and 46 % AT PK and TMK homes, respectively (Fig. 1). One or more intestinal parasites were observed in all 3 orphanages. The prevalence of single

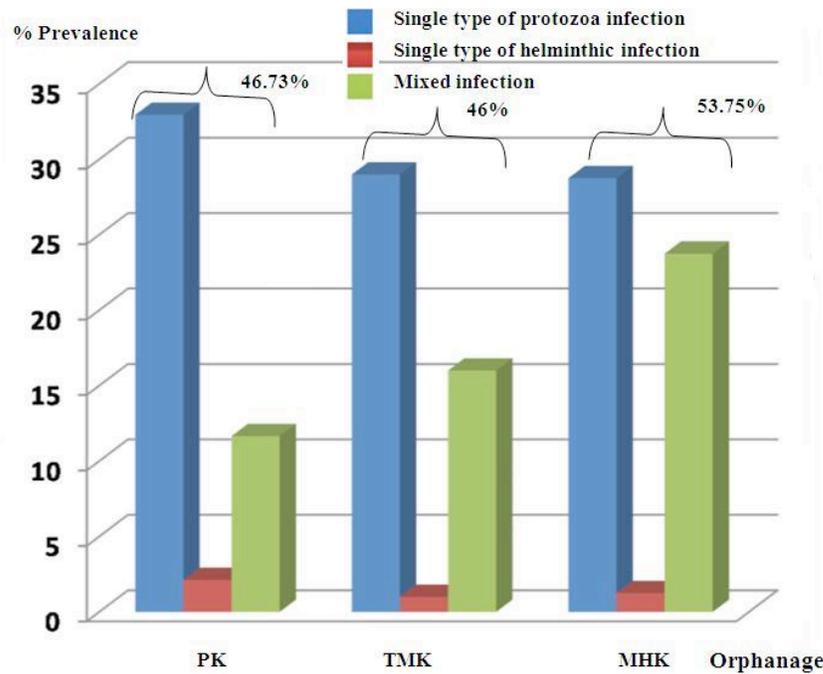


Fig. (1). Prevalence of intestinal parasitic infections classified by orphanages (PK, TMK and MHK, respectively). Overall intestinal parasitic infection was 133 (48.54%) out of 274 cases. Note that protozoa infection was much higher than helminthic infection.

protozoa infection was much higher than helminthes in all 3 orphanages (Fig. 1).

There were single and mixed intestinal parasitic infections in this study as shown in Table 1. However, the prominent one was a single infection presented at 33 (35.07%), 30 (30%) and 24 (30%) cases in PK, TMK and MHK homes, whereas mixed infections were 11 (11.66%), 16 (16%) and 19 (23.75%) cases in PK, TMK and MHK homes, respectively. When focusing only on protozoa, infected children with a single infection of protozoa were 31 (32.95%), 29 (29%) and 23 (28.75%) cases in PK, TMK and MHK homes, and helminthes were 2 (2.12%), 1 (1%) and 1 (1.25%) cases in PK, TMK and MHK homes, respectively. The prevalence of *G. duodenalis* infection in PK, TMK, and MHK homes was 10.63%, 12% and 15%, respectively (Fig. 2). Children living in MHK home demonstrated the highest prevalence of *G. duodenalis*. Other intestinal parasites included *Blastocystis hominis*, *Entamoeba coli*, *Endolimax nana*, *Entamoeba histolytica*-like, *Chilomastix mesnili*, *Trichuris trichiura*, *Hymenolepis nana*, *Strongyloides stercoralis* and Hookworm. The most frequent protozoa found in this study were *G. duodenalis* and *B. hominis*.

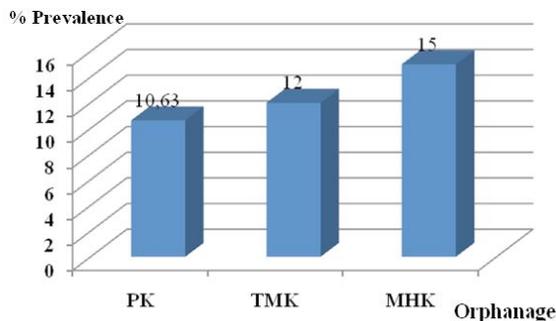


Fig. (2). Prevalence of *Giardia duodenalis* infection classified by orphanages (PK, TMK and MHK, respectively).

There were 14 out of 20 *Giardia* positive stool samples amplified by the 117 bp *ssrRNA* PCR products (Fig. 3), while 4 (20 %) were positive by the 432 bp *gdh* PCR products (Fig. 4A).

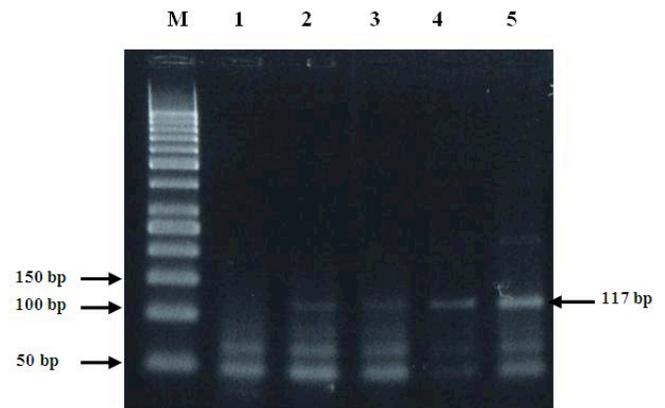


Fig. (3). 117 bp PCR products of *ssrRNA* gene of *Giardia duodenalis*. Lane M: 50 –bp ladder; lane 1: Negative control; lane 2-4: Positive unknown samples; lane 5: Positive control.

The 4 positive stool samples of 432 bp PCR products of *gdh* gene were digested with *Nla* IV and we found one stool sample that indicated sub-assembly AII; which showed a diagnostic genotyping profile (bp) as follows: 70, 80, 90, 120 (Fig. 4B, unknown sample No. 1). The remaining three stool samples belonged to the assemblages B, and showed a diagnostic genotyping profile (bp) as follows: 120 and 290, respectively (Fig. 4B, unknown sample No. 2-4). Assembly B was then further distinguished by *Rsa* I endonuclease into two sub-assemblages BIII and BIV. The predicted restriction fragment pattern showed that the remaining three stool samples indicated sub-assembly BIV, diagnostic

Table 1. Type of Intestinal Parasites Detected in 274 Thai Boy Orphans in 3 Orphanages

Types of Parasites	Orphanages (Homes)		
	PK n = 94 (%)	TMK n = 100 (%)	MHK n = 80 (%)
Single Infection			
Protozoa			
<i>G. duodenalis</i>	6 (6.38)	10 (10)	5 (6.25)
<i>B. hominis</i>	12 (12.76)	4 (4)	4 (5)
<i>E. coli</i>	2 (2.12)	10 (10)	6 (7.5)
<i>E. nana</i>	9 (9.57)	3 (3)	8 (10)
<i>C. mesnili</i>	0 (0)	1 (1)	0 (0)
<i>E. histolytica like</i>	2 (2.12)	1 (1)	0 (0)
Total	31 (32.95)	29 (29)	23 (28.75)
Helminthes			
<i>H. nana</i>	1 (1.06)	1 (1)	0 (0)
<i>S. stercoralis</i>	0 (0)	0 (0)	0 (0)
Hookworm	0 (0)	0 (0)	1 (1.25)
<i>T. trichiura</i>	1 (1.06)	0 (0)	0 (0)
Total	2 (2.12)	1 (1)	1 (1.25)
Mixed Infections			
Protozoa			
<i>G. duodenalis</i> + <i>B. hominis</i>	1 (1.06)	1 (1)	1 (1.25)
<i>G. duodenalis</i> + <i>E. coli</i>	1 (1.06)	0 (0)	2 (2.5)
<i>G. duodenalis</i> + <i>E. nana</i>	1 (1.06)	1 (1)	3 (3.75)
<i>G. duodenalis</i> + <i>E. nana</i> + <i>E. histolytica like</i> + <i>E. coli</i>	0 (0)	0 (0)	0 (0)
<i>G. duodenalis</i> + <i>E. nana</i> + <i>E. coli</i>	0 (0)	0 (0)	1 (1.25)
<i>B. hominis</i> + <i>G. duodenalis</i> + <i>E. nana</i> + <i>E. histolytica like</i>	0 (0)	0 (0)	0 (0)
<i>B. hominis</i> + <i>E. nana</i>	2 (2.12)	1 (1)	0 (0)
<i>B. hominis</i> + <i>E. nana</i> + <i>E. histolytica like</i>	1 (1.06)	0 (0)	0 (0)
<i>B. hominis</i> + <i>E. histolytica like</i>	1 (1.06)	0 (0)	0 (0)
<i>B. hominis</i> + <i>E. coli</i>	1 (1.06)	3 (3)	1 (1.25)
<i>E. histolytica like</i> + <i>E. coli</i>	2 (2.12)	0 (0)	1 (1.25)
<i>B. hominis</i> + <i>E. histolytica like</i> + <i>E. coli</i>	0 (0)	0 (0)	1 (1.25)
<i>E. nana</i> + <i>E. histolytica like</i> + <i>E. coli</i>	0 (0)	0 (0)	1 (1.25)
<i>B. hominis</i> + <i>E. nana</i> + <i>E. coli</i>	0 (0)	2 (2)	1 (1.25)
<i>E. nana</i> + <i>E. coli</i>	0 (0)	8 (8)	2 (2.5)
<i>E. nana</i> + <i>E. histolytica like</i>	0 (0)	0 (0)	1 (1.25)
Helminthes			
Hookworm + <i>S. stercoralis</i>	0 (0)	0 (0)	0 (0)
Protozoa + Helminthes			
<i>G. duodenalis</i> + <i>B. hominis</i> + <i>T. trichiura</i>	1 (1.06)	0 (0)	0 (0)
Hookworm + <i>S. stercoralis</i> + <i>E. nana</i>	0 (0)	0 (0)	1 (1.25)
Hookworm + <i>B. hominis</i>	0 (0)	0 (0)	1 (1.25)
Hookworm + <i>E. coli</i>	0 (0)	0 (0)	1 (1.25)
Hookworm + <i>E. coli</i> + <i>E. nana</i>	0 (0)	0 (0)	1 (1.25)

genotyping profile (bp) was as follows: 430 (BIV). Moreover, the sequencing analysis was confirmed the RFLP analysis (data not shown).

DISCUSSION

The findings in our study confirmed previous existing reports in Thailand [19-21], which show a high prevalence of

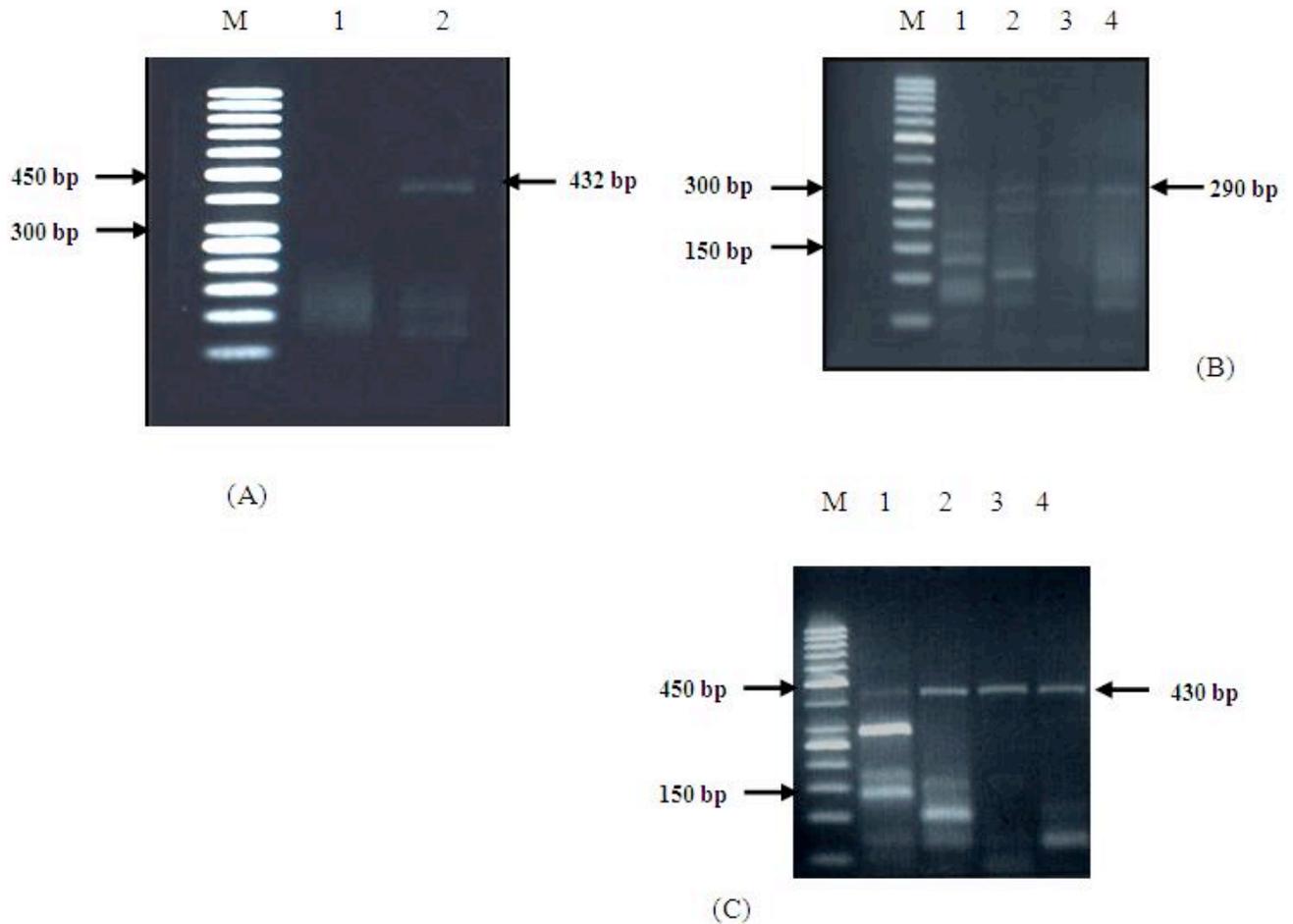


Fig. (4). PCR products of *gdh* gene of *G. duodenalis*. (A) Lane M: 50 –bp ladder; lane 1: Negative control; lane 2: *G. duodenalis* positive control. (B) RFLP analysis for 432 bp PCR products of *gdh* gene digested with the *Nla* IV. Lane M: 50 –bp ladder; lane 1-4, Unknown samples (1= Sub-assemblage AII, 2-4 = Assemblages B). (C) RFLP analysis for 432 bp PCR products of *gdh* gene digested with the *Rsa* I. Lane M: 50 –bp ladder; lane 1-4: Unknown samples. (1= Sub-assemblage AII, 2-4 = Sub-assemblage BIV).

intestinal parasitic infections. When focusing only on protozoa, especially *Giardia* and *Blastocystis* infections, they clearly show a higher infection rate than helminthes (Table 1). An improvement in personal hygiene, better sanitation and a widespread health education campaign have reduced helminthic infections, but protozoa are still present even in asymptomatic hosts. If attention is focused on pathogenic organisms, *G. duodenalis* predominates. Clinical presentations of giardiasis vary from asymptomatic carriage to acute and chronic diarrhea. Our study samples were healthy and usually needed no treatment. However, about half of asymptomatic *Giardia*-infected children excreted cysts for more than six months [22]. Therefore, they were potential carriers who could spread pathogens, including *Giardia*, to other persons in orphanages or communities while unaware that they were doing so. This is one explanation of why giardiasis is still prevalent worldwide and difficult to control.

The various prevalence of giardiasis in each area depends on personal and community hygiene. In general, giardiasis is more common in the developing world than in developed countries. Findings in stool specimens suggest the prevalence of *Giardia duodenalis* in the developing world was 20-30%, while in the industrialized world it was 2-5% [4]. It is more common in children living in orphanages than

those in day care centers (41.4% vs. 3.7%) [23]. In the present study, the prevalence of giardiasis in MHK home was higher than PK and TMH home. This might be because the MHK orphanage is located in a rural area, thus children living in this orphanage were closer to contaminated environments that served as potential sources of *G. duodenalis*. The other two orphanages are in urban locations that have better sanitation and where better standards of personal hygiene are practiced.

There are two major assemblages, including A and B, which can be recovered from human subjects whereas the assemblages C to G are specifically found in other mammalian hosts [24]. Sub-assemblage AI is commonly found in zoonotic pathogens, whereas sub-assemblage AII is restricted to humans. Furthermore, assemblage B appears to be human-specific [2]. The correlation between human clinical giardiasis and genotypes has been explored by many researchers. However, results were inconclusive. A study from Egypt denied any correlation between *G. duodenalis* genotype and clinical presentation [25]. Studies from Bangladesh, Peru and Spain showed a relationship between assemblage A, both sub-assemblages AI and AII, and diarrheal symptoms, while Assemblage B was found in asymptomatic hosts [26-28]. However, symptomatic giardiasis

related to Assemblage B was also demonstrated by studies from Saudi Arabia and Cuba [29, 30]. Children with sub-assemblage AII alone, or mixed infections with assemblage B, had diarrhea significantly more frequently ($P = 0.07$). In our study, children infected with sub-assemblage BIV isolate are more common and asymptomatic. Yet their treatment is neglected, leading to nutritional disorders and poor growth. Moreover, they easily spread *G. duodenalis* cysts in their environments.

We used *ssrRNA* and *gdh* genes for screening and genotyping *G. duodenalis*. The *ssrRNA* gene has high copy numbers arranged in tandem repeats as well as a more conserved sequence [31]. For these reasons, it is commonly used for screening *Giardia* infection in populations. Furthermore, the *gdh* gene was used on the basis of being able to differentiate genetic assemblages using *NlaIV* and *RsaI* endonuclease enzymes in PCR-RFLP analysis [27].

In asymptomatic orphans, we found sub-assemblage BIV more often than Assemblage A. However, the sample was too small and could not be amplified. Our findings revealed the same direction as Ratanapo and colleagues' study which was carried out in a different setting: school pupils in rural communities in Thailand [32]. Silent giardiasis children have no obvious symptoms, but they are still shedding cysts in their stool and are the source of infection in their immediate environment.

We, therefore, favor treating asymptomatic cyst passers so as to get rid of a potential source of infection in communities. Actively identifying silent giardiasis cases using highly sensitive tools is another important strategy. In addition, instruction in personnel hygiene and good sanitation along with proper health education in parasitic prevention should be continuously given to guardians and children. The benefits of these actions will be the control of human giardiasis and a reduction in public health problems.

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CONFLICT OF INTEREST

None Declared.

REFERENCES

- Caccio SM, Thompson RC, McLauchlin J, Smith HV. Unravelling *Cryptosporidium* and *Giardia* epidemiology. *Trends Parasitol* 2005; 21: 430-7.
- Almeida AA, Delgado ML, Soares SC, et al. Genotype analysis of *Giardia* isolated from asymptomatic children in northern Portugal. *J Eukaryot Microbiol* 2006; 53: S177-8.
- Adam RD. Biology of *Giardia lamblia*. *Clin Microbiol Rev* 2001; 14: 447-75.
- Ortega YR, Adam RD. *Giardia*: overview and update. *Clin Infect Dis* 1997; 25: 545-9.
- Farthing MJ. Giardiasis. *Gastroenterol Clin North Am* 1996; 25: 493-515.
- Fraser D, Bilenko N, Deckelbaum RJ, Dagan R, El-On J, Naggan L. *Giardia lamblia* carriage in Israeli Bedouin infants: risk factors and consequences. *Clin Infect Dis* 2000; 30: 419-24.
- Stuart JM, Orr HJ, Warburton FG, et al. Risk factors for sporadic giardiasis: a case-control study in southwestern England. *Emerg Infect Dis* 2003; 9: 229-33.
- Waikagul J, Krudsood S, Radomyos P, et al. A cross-sectional study of intestinal parasitic infections among schoolchildren in Nan Province, Northern Thailand. *Southeast Asian J Trop Med Public Health* 2002; 33: 218-23.
- Sirivichayakul C, Pojjaroen-anant C, Wisetsing P, Siripanth C, Chanthavanich P, Pengsaa K. Prevalence of intestinal parasitic infection among Thai people with mental handicaps. *Southeast Asian J Trop Med Public Health* 2003; 34: 259-63.
- Mungthin M, Suwannasaeng R, Naaglor T, Areekul W, Leelayoova S. Asymptomatic intestinal microsporidiosis in Thai orphans and child-care workers. *Trans R Soc Trop Med Hyg* 2001; 95: 304-6.
- Sakirirampant W, Nuchprayoon S, Wiwanitkit V, Yenthakam S, Ampavasi A. Intestinal parasitic infestations among children in an orphanage in Pathum Thani province. *J Med Assoc Thai* 2003; 86: S263-70.
- Wongstitwilairoong B, Srijan A, Serichantalergs O, et al. Intestinal parasitic infections among pre-school children in Sangkhlaburi, Thailand. *Am J Trop Med Hyg* 2007; 76: 345-50.
- Volotao AC, Costa-Macedo LM, Haddad FS, Brandao A, Peralta JM, Fernandes O. Genotyping of *Giardia duodenalis* from human and animal samples from Brazil using beta-giardin gene: a phylogenetic analysis. *Acta Trop* 2007; 102: 10-9.
- Solaymani-Mohammadi S, Singer SM. *Giardia duodenalis*: the double-edged sword of immune responses in giardiasis. *Exp Parasitol* 2010; 126: 292-7.
- Thompson RC, Monis PT. Variation in *Giardia*: implications for taxonomy and epidemiology. *Adv Parasitol* 2004; 58: 69-137.
- Amar CF, Dear PH, Pedraza-Diaz S, Looker N, Linnane E, McLauchlin J. Sensitive PCR-restriction fragment length polymorphism assay for detection and genotyping of *Giardia duodenalis* in human feces. *J Clin Microbiol* 2002; 40: 446-52.
- Radomyos P, Tangtrongchitr A, Krudsood S, Wilairatana P, Popruk S. Atlas of medical parasitology: with 465 colour illustrations. 8 ed. Bangkok: Medical media; 2010.
- Read CM, Monis PT, Thompson RC. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. *Infect Genet Evol* 2004; 4: 125-30.
- Sabchareon A, Chongsuphajaisiddhi T, Attanath P. Treatment of giardiasis in children with quinacrine, metronidazole, tinidazole and ornidazole. *Southeast Asian J Trop Med Public Health* 1980; 11: 280-4.
- Pengsaa K, Sirivichayakul C, Pojjaroen-anant C, Nimnual S, Wisetsing P. Albendazole treatment for *Giardia intestinalis* infections in school children. *Southeast Asian J Trop Med Public Health* 1999; 30: 78-83.
- Nuchprayoon S, Siriyasatien P, Kraivichian K, Porksakorn C, Nuchprayoon I. Prevalence of parasitic infections among Thai patients at the King Chulalongkorn Memorial Hospital, Bangkok, Thailand. *J Med Assoc Thai* 2002; 85: S415-23.
- Pikiewicz-Koch A. The frequency of giardiasis in various children's environment. *Przegl Epidemiol* 1999; 53: 339-43.
- Wongjindanon N, Suksrichavalit T, Subsutti W, Sarachart T, Worapisuttiwong U, Norramatha P. Current infection rate of *Giardia lamblia* in two provinces of Thailand. *Southeast Asian J Trop Med Public Health* 2005; 36: 21-5.
- Thompson RC. The zoonotic significance and molecular epidemiology of *Giardia* and giardiasis. *Vet Parasitol* 2004; 126: 15-35.
- Abdel-Moneim SM, Sultan DM. Genetic characterization of *Giardia lamblia* isolates from Egyptian patients with relation to clinical giardiasis. *J Egypt Soc Parasitol* 2008; 38: 547-60.
- Haque R, Mondal D, Karim A, et al. Prospective case-control study of the association between common enteric protozoal parasites and diarrhea in Bangladesh. *Clin Infect Dis* 2009; 48: 1191-7.
- Perez CG, Cordova PSO, Vargas VF, et al. Prevalence of enteroparasites and genotyping of *Giardia lamblia* in Peruvian children. *Parasitol Res* 2008; 103: 459-65.

- [28] Sahagun J, Clavel A, Goni P, *et al.* Correlation between the presence of symptoms and the *Giardia duodenalis* genotype. *Eur J Clin Microbiol Infect Dis* 2008; 27: 81-3.
- [29] Al-Mohammed HI. Genotypes of *Giardia intestinalis* clinical isolates of gastrointestinal symptomatic and asymptomatic Saudi children. *Parasitol Res* 2011; 108(6): 1375-81.
- [30] Pelayo L, Nunez FA, Rojas L, *et al.* *Giardia* infections in Cuban children: the genotypes circulating in a rural population. *Ann Trop Med Parasitol* 2008; 102: 585-95.
- [31] Nantavisai K, Mungthin M, Tan-ariya P, Rangsinsin R, Naaglor T, Leelayoova S. Evaluation of the sensitivities of DNA extraction and PCR methods for detection of *Giardia duodenalis* in stool specimens. *J Clin Microbiol* 2007; 45: 581-3.
- [32] Ratanapo S, Mungthin M, Soontrapa S, *et al.* Multiple modes of transmission of giardiasis in primary schoolchildren of a rural community, Thailand. *Am J Trop Med Hyg* 2008; 78: 611-5.

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