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Original research article

Prevalence and Genotype of *Giardia duodenalis* of Asymptomatic Individual in a Child Care Center, Bangkok, Thailand

Duangnate Pipatsatitpong[1*], Ratchaneewan Aunpad[1]
[1]*Faculty of Allied Health Sciences, Thammasat University, Pathum Thani 12120, Thailand*

Suradej Siripattanapipong[2]
[2]*Department of Microbiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

Mathirut Mungthin[3], Saovanee Leelayoova[3]
[3]*Department of Parasitology, Phramongkutklao College of Medicine, Bangkok 10400, Thailand*

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ABSTRACT

Giardia duodenalis (*G. duodenalis*) is an intestinal flagellate protozoan, which infects in humans and animals. It can be causing giardiasis. The symptoms are acute or chronic diarrhea, abdominal pain, weight loss and dehydration. *G. duodenalis* can be transmitted by eating food or drinking water contaminated with infected stage cysts. Children who live and share facility in an orphanage are the high risk groups for infection. The objective of this paper is to study the prevalence and genotypes of *G. duodenalis* infection of asymptomatic individual in a child care center. This study was conducted in Mercy Centre orphanage, Klong Toey community. 289 stool samples were collected from children and childcare workers. Stool samples were extracted DNA. Small subunit ribosomal DNA (SSU-*rDNA*) and glutamate dehydrogenase (*gdh*) genes were amplified using Nested PCR. PCR products of *gdh* gene were cut using *Nla*IV and *Rsa*I restriction enzymes. Genotypes were identified. The results of this study showed the prevalence of giardiasis in an orphanage, Klong Toey community, Thailand was 17.6% (51/289). PCR-RFLP analysis of 19 samples revealed that genotype AII was 63.2% (12/19), genotype BIV was 31.6% (6/19) and genotype BIII was 5.3% (1/19) respectively. Consequently, the study of prevalence and genotypes of *G. duodenalis* is beneficial for control planning of giardiasis in an orphanage, Klong Toey community.

***Corresponding author:** duangnat@tu.ac.th **doi:** 10.14456/scitechasia.2018.20

Keywords: *Giardia duodenalis*; Genotype; Assemblage

1. Introduction

Giardia duodenalis (*G. duodenalis*) is an intestinal flagellate protozoan, causing giardiasis, which infects a wide range of host, such as humans, domestic animals. *G. duodenalis* is one of the most intestinal protozoan that distributes worldwide [1-3]. The prevalence of *G. duodenalis* is found in all age and children are the greatest risk group for infection. The prevalences of *G. duodenalis* infection range from 1.3% to 37.7% in children, Thailand [4-7]. Infection occurs by ingestion of viable cysts, which are transmitted by fecal-oral route, person to person or animal to person, water borne and food borne. The symptoms produce acute or chronic diarrhea, malabsorption, weight loss and abnormal growth in children [1-3]. *G. duodenalis* has eight major genotypes, genotype A-H. Genotype A and B are associated with humans. Other animal-specific groups include genotype C, D, E, F and G. Genotype A has two distinct clusters, AI and AII, while genotype B consists of BIII and BIV [1-3]. The genotypes infected in Thailand are sub-genotype such as BIV, AII, BIII and AI respectively. Nowadays, the common identify method of *G. duodenalis* is microscopic method in direct stool smear. However, microscopic method is high specificity and easily to identify but it has low sensitivity. Immunofluorescent method is superior sensitivity but it is also more expensive. These techniques might not be sensitive enough to detect low number of *Giardia* cysts and cannot be used to discriminate genotype of *G. duodenalis*. Therefore, nested polymerase chain reaction (Nested PCR) method has been developed. It uses 2 sets of primer that aims to increase specificity and number of PCR products. Nested PCR method uses for diagnosis which is higher sensitivity than microscopic method and immunofluorescent method. Because it can detect a low number of *Giardia* cysts [3]. PCR-RFLP technique uses for determine genotype and sub-genotype of *G. duodenalis*. Furthermore, PCR-RFLP can be clearing to analyze mix genotypes. In Thailand, many study of *G. duodenalis* were conducted only in some especially rural community such as Chacheongsao province and Mae-chaem, Hod districts, Chiang Mai province [5, 7]. At the present time, information about prevalence and genotype of giardiasis in urban community of Thailand are not available. Consequently, the objectives of this study were determined the prevalence and the genotype of *G. duodenalis* in an orphanage, Klong Toey community.

2. Materials and Methods

2.1 Stool collection

Two hundred and eighty nine stool specimens were collected from children and child care workers in the Mercy Centre orphanage, Klong Toey community during September 2009 to September 2010, participants were voluntarily enrolled into the previous study, with the written informed consent from their parents or a head of child care institute. Personal data of children was provided by their parents or child care workers. All stool specimens were kept at -20°C without preservative agent. Research protocol in this study was approved by the Ethics Committee of Thammasat University.

2.2 DNA extraction

All stool samples were extracted DNA using QIAmp DNA stool mini kit (QIAGEN, Germany) according to manufacturer instructions [8].

2.3 Genotypic characterization of *Giardia duodenalis*

Genotypic characterization of *G. duodenalis* was determined using amplification of the small subunit ribosomal

gene (SSU-*rRNA*) as previously described by Hopkins RM, et al. [9].

PCR-RFLP was also performed using nested PCR of 461 bp region of the glutamate dehydrogenase (*gdh*) gene. The primary primer pairs (forward primers; GDH1, GDH1a and reverse primer; GDH5s) with the condition for the first round PCR were used as previously described by Boontanom P, et al., 2011 [5]. The secondary PCR, forward primer GDHeF and reverse primer GDHiR were used and the condition was described by Read CM, et al. 2004 [3]. To identified genotype and sub-genotype of *G. duodenalis*, a 461 bp of the *gdh* gene was digested with restriction enzyme *Nla*IV to differentiate all major genotype including sub-genotype AI and AII. A restriction enzyme *Rsa*I was used to distinguish between sub-genotype BIII and BIV. PCR products and restriction fragments were separated by electrophoresis in 1.5% agarose gel, Gels were stained with ethidium bromide and visualized under UV light and documented by using a gel documentation system. All positive PCR product samples were confirmed by DNA sequencing (AIT Biotech Company). The genotype of *G. duodenalis* from each specimen was confirmed based on the homology of the sequenced PCR product to the published sequence in GenBank.

3. Results and Discussion

3.1 Results

A total of 289 stool samples from an orphanage, Klong Toey community Thailand were analyzed by nested PCR using amplification of the SSU-*rDNA* gene and *gdh* gene. Using RH11/RH4 as primary primers and GiarF/GiarR as secondary primers for SSU-*rDNA* gene (Figure 1), 51 samples (17.6%) were positive results. Using *GDH*1/*GDH*1a/*GDH*5s as primary primers and *GDH*eF/*GDH*iR as secondary primers for *gdh* gene (Figure 2), 19 samples (6.6%) were positive results. For diagnostic genotyping, *gdh* PCR products could be used RFLP analysis by *Nla*IV restriction enzyme for AI, AII genotype identifications and *Rsa*I restriction enzyme for identified BIII and BIV genotypes (Figure 3-4). Of 19 samples, 12 samples (63.2%) were classified into genotype AII, 1 sample (5.3%) was genotype BIII, 6 samples (31.6%) were genotype BIV, whereas genotype AI was not found.

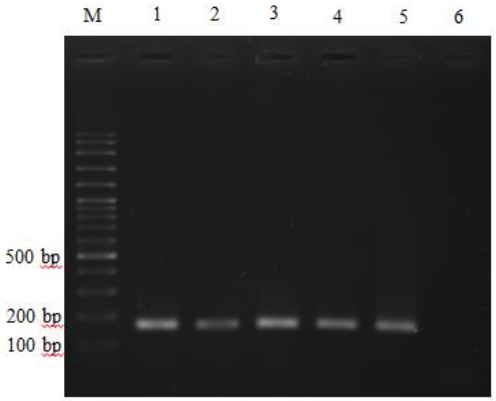

Fig.1. Nested PCR amplification of *Giardia duodenalis* SSU-*rDNA* gene.

Lane M; 100 bp markers, lane 1-4; stool samples, lane 5; positive control, lane 6; negative control.

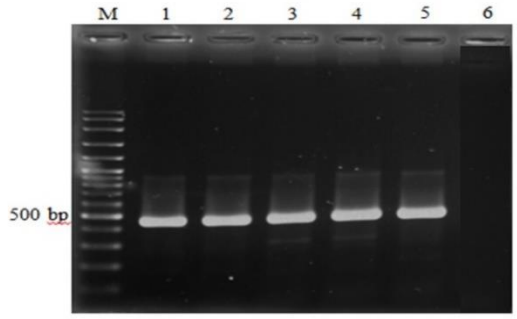

Fig.2. Nested PCR amplification of *G. duodenalis*; *gdh* gene.

Lane M; 100bp markers, lane 1-4; stool samples; lane 5; positive control, lane 6; negative control.

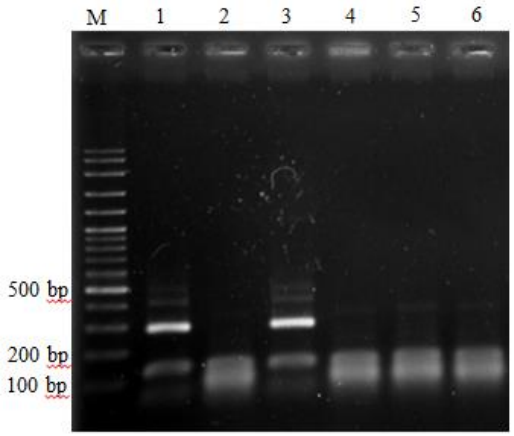

Fig.3. RFLP analysis using *Nla*IV restriction enzyme.

Lane M; 100 bp markers, lane 1 and 3; Suspected B genotype, lane 2, 4-6; AII genotype.

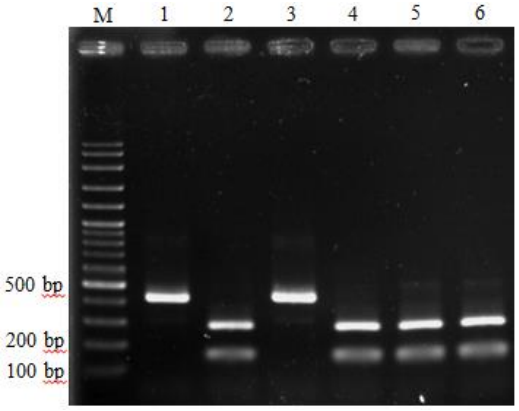

Fig.4. RFLP analysis using *Rsa*I restriction enzyme.

Lane M; 100 bp markers, lane 1 and 3; BIV genotype, lane 2, 4-6; BIII genotype.

3.2 Discussion

The prevalences of giardiasis of the children population in Thailand were previously reported in many studies. The prevalence of giardiasis in children varies from 1.3 to 37.7%. [4-7]. In 2008, a study in primary school of a rural community, Chacheongsao province showed a prevalence of 6.2% [6]. Next, in 2011, a study in preschool at Sanamchaiket District, Chacheongsao province showed a prevalence of 5.8% [5]. The last, in 2012, a study in hilltribe children, Mae-chaem and Hod districts, Chiang Mai showed a prevalence of 5.2% [9]. However, the present study showed the prevalence of orphanage was 17.6%. The high prevalence not only depended on sanitary living place and their hygiene but also depended on sensitivity of the identify methods, Molecular technique such as conventional PCR, nested PCR have higher sensitivity than microscopic method.

The genotypes of *G. duodenalis* infection in humans were genotype A (AI, AII) and B (BIII, BIV). In 2011, a study in preschool children at Sanamchaiket District, Chacheongsao province showed the most frequent genotype of *G. duodenalis* was BIV, followed by AII and BIII respectively whereas genotype AI was not found [5]. This result was the same as the study in primary schoolchildren of a rural community, Chacheongsao province [6] and the study in hilltribe children, Hod district, Chiang Mai [7]. The study in hilltribe children, Mae-chaem district, Chiang Mai showed the most frequent genotype was AII, followed by BIV whereas genotype AI and BIII were not found. However, this study showed the most frequent genotype was AII, followed by BIV and BIII respectively whereas genotype AI was not found.

In 2014, Boontanom P, et al. reported the genotypes of *Giardia. duodenalis* from stool sample freezing. Stool samples were collected from children in an orphanage in suburban area outside Bangkok, Central Thailand during January 2007 to January 2008 [10]. Genotype AII was the most predominant found, followed by genotype BIV in an orphanage cohort.

During January 2015 to August 2018 [11-13], many studies investigated parasite infections among children in Thailand [11-13], *G. duodenalis* was still a highlight protozoan infection among children.

However, there have no report of genotypes determination.

Comparison of the diagnostic tests; such as microscopy, ELISA and PCR for the detection of *G. duodenalis*. The sensitivity of the PCR technique was higher than the ELIZA and microscopy technique [14]. PCR-RFLP technique can be used to discriminate genotype of *G. duodenalis*.

4. Conclusion

The prevalence of giardiasis in an orphanage, Klong Toey community was 17.6%. PCR-RFLP analysis of 19 stool samples revealed that genotype AII (63.2%), BIV (31.6%) and BIII (5.3%) were found, whereas genotype AI was not found. This information would be help to develop a treatment planning, preventing and controlling of giardiasis in an orphanage, Klong Toey community, Thailand.

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