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Epidemiology of giardiasis and genotypic characterization of *Giardia duodenalis* in preschool children of a rural community, central Thailand

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Abstract. A cross-sectional study was conducted to determine the prevalence and risk factors of *Giardia duodenalis* infection in 189 preschool children at Sanamchaiket District, Chachoengsao Province, central Thailand in February 2007. Stool specimens were examined for the presence of *Giardia* using simple wet preparation and PBS-ethyl acetate concentration technique. The prevalence of *G. duodenalis* in preschool children was 5.8%. Using PCR-RFLP analysis of the glutamate dehydrogenase gene (*gdh*), genotypes of *G. duodenalis* revealed assemblage AII (3, 30.0%), BIII (1, 10.0%), and BIV (6, 60.0%). Using multivariate analysis, children who kept cat(s) at home were at 5.1 times (95% CI; 1.3-20.3) greater risk of acquiring giardiasis. This study possibly represents the information supporting the potential zoonotic transmission of *G. duodenalis* between cats and preschool children. Unfortunately, in this study, we did not determine *G. duodenalis* infection in cats, so further studies in cats should be performed to confirm this postulation.

INTRODUCTION

Giardia duodenalis, the causative agent of giardiasis, is one of the most frequently diagnosed intestinal protozoa infections reported worldwide (WHO, 1996; Thompson, 2004). Prevalence of *G. duodenalis* is found in all age groups, but children are at the greatest risk for contracting clinical giardiasis, especially those attending day care centers (Polis *et al.*, 1986), living in poor hygienic conditions (Saksirisampant *et al.*, 2003) or in community settings (Ratanapo *et al.*, 2008). Infection occurs by the ingestion of viable cysts, which are transmitted through fecal-oral contamination by direct person-to-person, water-borne transmission, food-borne transmission, and animal-to-person transmission. The infection may produce severe acute diarrhoea in young children and impairs body weight gain, interfering with growth and development (Wolfe, 1992).

Genotypic characterization of *G. duodenalis* has been shown to be a useful tool in epidemiological studies or outbreak investigations (Robertson *et al.*, 2006). Molecular studies have revealed a complex species of G. duodenalis from which two major genetic assemblages, A and B, are recovered from humans (Mayrhofer *et al.*, 1995; Monis *et al.*, 1996, 1999, 2003). Other animal-specific groups include assemblages C, D, E, F, and G (Monis *et al.*, 1999, 2003). Assemblage A has two distinct clusters; AI and AII while assemblage B consists of BIII and BIV (Monis *et al.*, 1996). Both assemblages A and B are also reported in domestic and wild animals (Monis *et al.*, 2003). Thus, potential zoonotic transmission to humans

of assemblages A and B is plausible. It has been shown that living in close contact with dogs was a significant risk factor of getting giardiasis in primary school children (Traub *et al*., 2004; Ratanapo *et al*., 2008). Using molecular tools, the potential zoonotic transmission of *G. duodenalis* from domestic dogs to humans was also demonstrated (Traub *et al*., 2004; Inpankaew *et al*., 2007). Although, genotypic characterization has been used to assess the role of zoonosis in human giardiasis, the epidemiological information supporting zoonotic transmission of G. duodenalis is still limited as the direct evidence has not been demonstrated.

Previous studies in Thailand have shown that the prevalence of giardiasis in children of different populations varies from 12 to 37.7% (Janoff *et al*., 1990; Mungthin *et al*., 2001; Saksirisampant *et al*., 2003; Ratanapo *et al*., 2008). However, most studies do not evaluate the risk factors of acquiring *G. duodenalis* infection which are essential for prevention and control strategies. In the present study, we conducted a cross-sectional study of giardiasis in preschool children to determine the prevalence and identify associated risk factors using standardized questionnaires. In addition, genotypic characterization of *G. duodenalis*, isolated from these children, was also investigated.

MATERIALS AND METHODS

Study design and study population

A cross-sectional study of intestinal parasites was conducted by survey in a preschool, located at Sanamchaiket District, Chachoengsao Province, central Thailand in February 2007 consisting of 225 preschool children aged between three and nine years old. The research protocol was approved by the Ethics Committee of the Royal Thai Army, Medical Department. Written informed consent was obtained from parents for children to participate in the study. A stool sample from each child was collected and examined for intestinal parasitic infection under light microscopy using simple wet preparation and PBS-ethyl acetate sediment concentration technique. Short-term cultivation using Jone's medium was performed to detect *Blastocystis hominis*. To collect positive *G. duodenalis* cysts for genotypic characterization, stool specimens were concentrated using the saturated sodium nitrate flotation technique. The concentrated cysts were washed three times with phosphate buffered saline and kept at -20°C until use.

DNA extraction of *G. duodenalis* in stool specimens

The DNA of *G. duodenalis* positive samples were extracted using FTA disk (Whatman, Bioscience, U.S.A.). Fifteen microliters of each concentrated specimen was applied onto a 6 mm-diameter FTA disk and left air-dried overnight. The disk was cut into four pieces and one piece was used for each test. The FTA disk was washed twice with 200 µl of FTA purification buffer (Life Technologies, Gaitherburg, MD.) for 15 min, then washed twice with 200 µl of TE buffer (10 mM Tris-HCl pH 8.0; 0.1 mM EDTA pH 8.0) for 5 min and dried overnight. The washed FTA disks were used as DNA templates in PCR amplification. In addition, the QIAamp stool mini kit (Qiagen, Germany) was used for DNA extraction for samples that gave negative PCR results after using FTA disk with final elutions of 100 µl instead of 200 µl as recommended by the manufacturer.

Genotypic characterization

Amplification of the *gdh* gene of *G. duodenalis* was carried out by nested PCR using specific primer pairs and PCR-RFLP analysis was used to identify genotypes. To amplify the gdh gene, a primary External Forward Primer, GDH1 (5'ATC TTC GAG AGG ATG CTT GAG3'), GDH1a (5'ATC TTC GAG AAG GAT GCT TGA G3'), and External Reverse Primer, GDH5s (5'GGA TAC TTS TCC TTG AAC TC3') were used for primary PCR assay (Boontanom *et al*., 2010). For the secondary PCR, a 461 bp of the gdh gene was amplified using GDHeF (5'TCA ACG TYA AYC GYG GYT TCC GT3')

and GDHiR (5'GTT RTC CTT GCA CAT CTC C3') with the condition previously described by Read *et al.* (2004). The first-round PCR amplification was performed using mixtures of 2 U of Taq polymerase with 1X PCR buffer, 2.5 mM $MgCl_2$, 250 mM of each dNTP, 25 pmol of each primer, and two FTA disks (or 1 to 2 µl of the extracted DNA) in a total volume of 50 µl. The thermal cycling conditions were: 94ºC for 7 min and then 35 cycles of 94ºC for 1 min, 55ºC for 1 min, and 72ºC for 1 min, with a final cycle of 72ºC for 7 min. The second-round PCR was performed using mixtures of 2U of Taq polymerase with 1X PCR buffer, 1.5 mM $MgCl_2$, 200 mM of each dNTP, 25 pmol of each primer, and 1 µl of the primary PCR product in a total volume of 50 µl. The thermal cycling was initiated with 1 cycle of 94ºC for 2 min, 56ºC for 1 min, and 72ºC for 2 min, followed by 55 cycles of 94ºC for 30 sec, 56ºC for 20 sec, 72ºC for 45 sec, and final extension at 72ºC for 7 min (Read *et al.*, 2004). The PCR products were analyzed by 2% agarose gel electrophoresis, stained with ethidium bromide and then visualized on a UV transilluminator. To identify assemblages and subgenotypes of *G. duodenalis*, a 461 bp of the *gdh* gene was digested with *Nla* IV endonuclease enzyme to differentiate all major assemblages including subgroups AI and AII. A restriction enzyme *Rsa*I was used to distinguish between assemblages BIII and BIV.

Questionnaires

To identify risk factors of *G. duodenalis* infection, standardized questionnaires, covering demographic data, sanitary behaviours including cooking and eating habits, sources and treatment methods of drinking water, pets or animal contact, and a history of present gastrointestinal symptoms, were used. The weight and the height of each child were recorded at school to determine their nutritional status using the Thai standard growth curve, Ministry of Public Health, Thailand, 1999. Parents of the enrolled children were asked to complete the questionnaires.

Statistical analysis

The association between potential risk factors and *G. duodenalis* infection was assessed by the Chi-square test with a 95% confidence interval. Odds ratios with 95% confidence interval and *p* values were calculated between *G. duodenalis* infection and each of the other variables in the bivariate analysis. Logistic regression was performed for multivariate analysis to assess the independent association of risk factors and giardiasis. All the analyses were conducted using Stata/SE for Windows version 9.2 (StataCorp LP, College Station, TX).

RESULTS

Prevalence of intestinal parasitic infections

Protozoa infections were the most predominant parasites detected in these preschool children (14.8%) (Table 1). *Blastocystis homonis* was the most common intestinal parasite (9.0%), followed by *G. duodenalis* (5.8%). Other helminthic infections were hookworm (1.6%), *Strongyloides stercoralis* (1.1%) and *Enterobius vermicularis* (0.5%). Twelve subjects (10.0%) experienced diarrhoea. Those who were infected with pathogenic parasites were treated with appropriate antiparasitic drugs.

Characteristics of the enrolled children

Of the 225 preschool children, 189 (84%) were enrolled in the study. The subjects comprised 95 males (50.3%) and 94

Table 1. Prevalence of intestinal parasitic infections in 189 preschool children

Intestinal parasitic infection	Number	Percent
Blastocystis hominis	17	9.0
Giardia duodenalis	11	5.8
Hookworm	3	1.6
Strongyloides stercoralis	2	1.1
Enterobius vermicularis	1	0.5
Total	34	18

females (49.7%), ranging in age between three and nine years old and the median age was five years. Thirty four (18.0%) preschool children were positive for intestinal parasitic infections.

Characteristics of the enrolled children are shown in Table 2. The 11 subjects positive for *G. duodenalis* comprised seven boys and four girls, aged between four and six years. The prevalence of *G. duodenalis* infection was not significantly different among age groups, classrooms or nutritional status. The highest prevalence of *G. duodenalis* infection (5, 7.6%) was found in four years old children, followed by five and six years old, respectively. Most infected children had no gastrointestinal symptoms during the study.

Risk factors of *G. duodenalis* infection

Univariate and multivariate analysis of risk factors associated with *G. duodenalis* infection are shown in Table 3. No significant association was found between *G. duodenalis* infection and age, sex, washing hands before meals, class room,

Table 2. Characteristics of preschool children enrolled in this study and the prevalence of *G. duodenalis* at preschool children, Sanamchaiket district, Chachoengsao Province, Thailand

Characteristics	Total (%)	No. positive for *G. duodenalis* (%)	*p*- value
Age (year)			
3	4 (2.2)	0 (0)	
4	52 (28.0)	4 (7.5)	
5	73 (40.7)	4 (6.5)	
6	49 (28.0)	2 (3.8)	
>6	6 (1.1)	0 (0)	0.447
Class room			
Pre-Kindergarten	12 (6.3)	1 (8.3)	
No. 1/1	14 (7.4)	1 (7.1)	
No. 1/2	10 (5.3)	1 (10.0)	
No. 1/3	14 (7.4)	0 (0)	
No. 1/4	8 (4.2)	1 (12.5)	
No. 2/1	18 (9.5)	2 (11.1)	
No. 2/2	13 (6.9)	1 (7.7)	
No. 2/3	16 (8.5)	1 (6.3)	
No. 2/4	18 (9.5)	1 (5.6)	
No. 3/1	16 (8.5)	1 (6.3)	
No. 3/2	16 (8.5)	1 (6.3)	
No. 3/3	17 (9.0)	0 (0)	
No. 3/4	17 (9.0)	0 (0)	0.953
Sex			
Male	95 (50.3)	7 (7.4)	
Female	94 (49.7)	4 (4.3)	0.361
Diarrhoea			
No	108 (90.0)	4 (3.7)	
Yes	12 (10.0)	2 (16.7)	0.110
Nutritional status			
Normal	82 (45.1)	3 (3.7)	
Low normal and under weight	100 (54.9)	8 (8.0)	0.350

Table 3. Univariate and multivariate analysis of risk factors of *G. duodenalis* infection

Characteristics	*G. duodenalis*		Crude odds ratio (95%CI)	*p*-value	Adjusted odds ratio** (95%CI)	*p*-value
	Negative	Positive				
Age group (years)						
>5	57(96.6)	2(3.4)	1		1	
3-5	117(93.6)	8(6.4)	1.9(0.4-9.5)	0.505	0.5(0.1-2.5)	0.371
Sex						
Female	90(95.7)	4(4.3)	1		1	
Male	88(92.6)	7(7.4)	1.8(0.5-6.3)	0.361	0.5(0.1-1.9)	0.285
Class group						
Pre-Kindertgarten	11(91.7)	1(8.3)	1			
Kindergarten	167(94.4)	10(5.6)	1.5(0.2-13.0)	0.524		
No. of children of age <6 years in home						
>2	49(96.1)	2(3.9)	1			
<2	99(92.5)	8(7.5)	2.0(0.4-9.7)	0.502		
Keeping dog(s) at home						
No	41(91.1)	4(8.9)	1			
Yes	109(94.8)	6(5.2)	0.6(0.2-2.1)	0.469		
Keeping cat(s) at home						
No	111(96.5)	4(3.5)	1		1	
Yes	40(87.0)	6(13.0)	4.2(1.1-15.5)	0.033	5.1(1.3-20.3)	0.021
Washing hands before meal						
Every time	50(98.0)	1(2.0)	1		1	
Occasionally	98(91.6)	9(8.4)	4.6(0.6-37.3)	0.169	0.3(0.1-2.1)	0.207
Drinking filtered and boiled water						
Yes	22(95.7)	1(4.3)	1			
No	129(94.2)	8(5.8)	1.4(0.2-11.5)	1.000		

**Adjusted for age group, sex, and washing hands before meal

number of children aged less than six years living in the same household, keeping a dog or dogs at home, and drinking filtered and/or boiled water. However, univariate analysis showed that children who kept a cat or cats at home were at 4.2 times greater risk of acquiring *G. duodenalis* (95% CI=1.1-15.5). After adjusting for age, sex, and washing hands before meals, multivariate logistic regression analysis showed that children who kept a cat or cats at home were at 5.1 times greater risk of acquiring *G. duodenalis* than those without cats at home (95% CI=1.3-20.3).

Genotypic characterization

Of the 11 *G. duodenalis* positive samples, PCR amplification of the *gdh* gene and SSU gene were successful in ten samples (90.9%). After using RFLP analysis, the results showed assemblage AII (3, 30.0%), BIII (1, 10.0%), and BIV (6, 60.0%), whereas assemblage AI was not found.

DISCUSSION

The prevalence of *G. duodenalis* infection in orphans in 1990 and 2001 was 20% and

12%, respectively (Janoff *et al.*, 1990; Mungthin *et al.*, 2001) of which 21% was reported in school children of low socioeconomic status (Chavalittamrong & Jirapinyo, 1984) and the highest prevalence of 37.7% was reported in an orphanage at baby's nursing home in Pathumthani Province, Central Thailand (Saksirisampant *et al.*, 2003). In the present study, the prevalence of *G. duodenalis* infection in preschool children was considered as low, compared with those reported in other children populations in Thailand. The low prevalence in this community might be due to their living conditions that these children were living in their own houses which might lower the risk of person-to-person transmission compared with those living in orphanages.

In this study, the prevalence of *G. duodenalis* was similar to many surveys of intestinal parasitic infections in Thailand (Ratanapo *et al.*, 2008) that it was the second most common, next to *B. hominis*, found in preschool children. However, the true prevalence of *G. duodenalis* in this study could have been underestimated since only microscope method was used without confirmation by more sensitive techniques, such as PCR.

The zoonotic transmission of *G. duodenalis* has gained more evidence, particularly the role of domestic animals. It has been reported that dogs can harbour infections with either zoonotic or host-specific assemblages of *Giardia* (Caccio *et al.*, 2002; Traub *et al.*, 2004; Inpankaew *et al.*, 2007; Thompson *et al.*, 2007). Few studies have been undertaken to support a potential zoonotic transmission from cats. Vasilopulos *et al.* (2007) examined 250 cats from Mississippi and Alabama, U.S.A of which 17 were positive for *Giardia* and revealed 6 were infected with subgenotype AI and 11 infected with specific assemblage F of cats. Souza *et al.* (2007) also revealed 19 samples from cats composed of 8 subgenotype AI and 11 assemblage F. In Italy, an isolate from a cat was identified as subgenotype AII (Caccio *et al.*, 2008). Moreover, subgenotype BIV has also been reported in cats in Australia (Read *et al.*, 2004). Although, the information of genotypic characterization of *G. duodenalis* in cats became evident, epidemiological data is still lacking. From our study, a significant association between *G. duodenalis* infection and keeping a cat or cats at home was identified which might emphasize the role of cats as a potential zoonotic transmission in this community. Unfortunately, we could not collect cat fecal specimens to confirm the presence of *G. duodenalis* and their assemblages. Thus, only qualitative analysis was done in this study. Direct evidence of the zoonotic transmission from cats to human still needs further studies.

In conclusion, the present study showed that assemblage B, subgenotype BIV was common in preschool children. Our study showed that keeping cats at home was a significant risk factor of acquiring *G. duodenalis* infection in preschool children of this community. This information will be useful to the effective prevention and control programme of giardiasis in this population.

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