J Trop Med Parasitol. 2014;37:35-41.

RESEARCH

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Prevalence of *Giardia duodenalis* and factors associated with its infection in water buffaloes in Northeast Thailand

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ABSTRACT

Giardia spp. is the intestinal protozoa causing giardiasis in animals and humans. Transmission of the disease occurs via the fecal-oral route between animals to animals and animals to humans. The objectives of this study were to determine the prevalence of *Giardia duodenalis*, identify the assemblage, and analyze factors associated with its infections of water buffaloes in Northeast Thailand. A total of 567 buffalo fecal samples were collected from northeast provinces including Sakon Nakhon, Buri Ram, Ubon Ratchathani, Roi Et, Si Sa Ket and Surin. Fecal samples underwent zinc sulphate floatation and nested PCR (nPCR) based on the SSU-rRNA gene. The overall prevalence of *Giardia duodenalis* infections by nPCR was 0.4% (2/567). The positive DNAs were sequenced and compared to reference nucleotide sequences from GenBank. The prevalence of *G. duodenalis* infections in water buffaloes among six provinces was ranged between 0 to 1.6%. Buri Ram had the highest individual infections (1.6%, 1/62) and the herd prevalence was 0.7% (2/276). Regarding the factors associated with *G. duodenalis* infections, sex, age, herd size, and geographical landscape (basin) were brought to analysis, but they were not statistically significant.

Keywords: Water buffaloes, *Giardia duodenalis*, PCR, Northeast Thailand

INTRODUCTION

Giardia duodenalis is an intestinal protozoan parasite frequently found in domestic animals and humans worldwide [1]. Currently, the molecular data based on protein and DNA polymorphisms have shown that *G. duodenalis* has eight distinct genetic groups or assemblages (A to H), i.e., Assemblages A and B in mammals including humans, Assemblages C and D in canine, Assemblage E in livestock, Assemblage F in feline,

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Assemblage G in rodents, and Assemblage H in marine mammals [2].

Transmission of *G. duodenalis* is by swallowing of viable cysts contaminated in food or drinking water either via fecal-oral route or contact between human-to-human (anthroponotic) or animal-to-human (zoonotic) [3]. Outbreaks of symptomatic human giardiasis in developing countries including Asia, Africa, and Latin America have affected more than 200 million people, and 500,000 new cases were annually reported [4]. It is predicted that, by 2050, more than 50% of people in these regions will be living in urban and suburban conditions; many of them living with poor hygiene, where *Giardia* would be easily transmitted [4].

The clinical signs of giardiasis frequently found in humans are diarrhea and weight loss which have led to increase morbidity and economic losses. However, giardiasis is commonly recognized as an asymptomatic infection in animals [3]. Giardiasis in animals, particularly in cattle is caused by *G. duodenalis* Assemblage E and zoonotic Assemblage A and/or B [5-7]. Therefore, cattle might be at potential risk for zoonotic transmission via direct contact or contaminated water or foods [8-9]. However, there were a few reports of *G. duodenalis* Assemblages E and A in buffaloes worldwide [10-11].

Water buffalo (*Bubalus bubalis*) is common in the Southeast Asian region since buffaloes are raised for agricultural practices and as meat product with integrated crop-livestock farming systems. They are grazing in nearby public or their own pasture so that they might pollute environment resulting in an impact on human or animal health [12]. Therefore, buffaloes might be regarded as a reservoir for some zoonoses including giardiasis. The objective of this study was to investigate the prevalence and factors associated with *G. duodenalis* infection in water buffaloes in northeast Thailand.

MATERIALS AND METHODS

1. Fecal samples collection

A total of 567 fecal samples were collected from buffaloes of 6 northeast provinces with the highest population consensus including Sakon Nakhon, Buri Ram, Ubon Ratchathani, Roi Et, Si Sa Ket, and Surin, during January to April 2010 (Figure 1). Each fecal sample (approximately 10 g) was directly collected from rectum of an individual buffalo by using disposable gloves. Fecal samples were stored in icebox, transported to Department

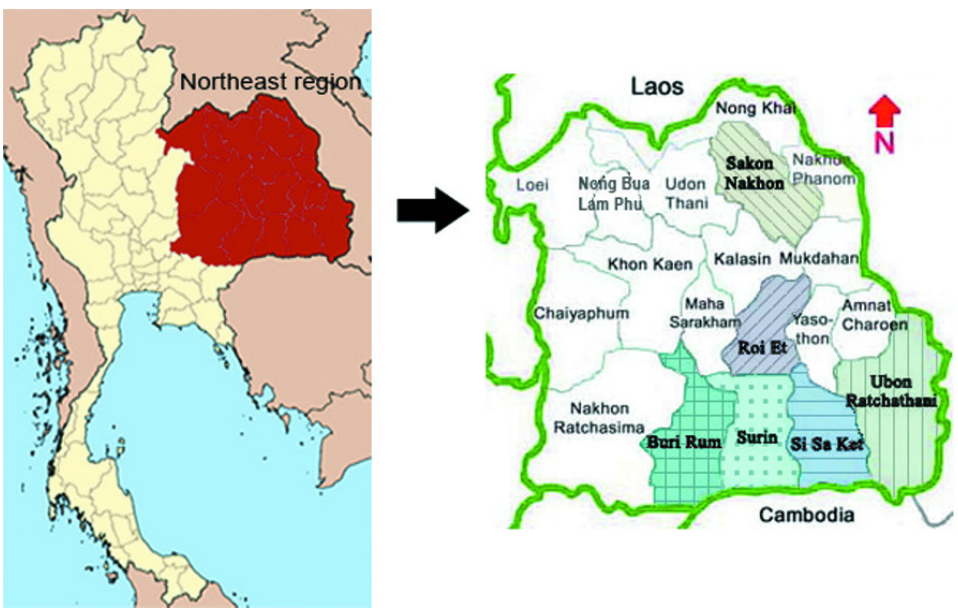

Fig. 1 Locations of fecal sample collection

of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand. Small portions of fecal sample were separately preserved in 10% formalin and 70% alcohol and left at room temperature while the rest was stored at -20˚C before processing for PCR.

2. Zinc sulphate floatation technique

Fecal samples in 10% formalin were mixed with 10 ml of Zinc sulphate solution (specific gravity = 1.18) and the tube was later on filled up with zinc sulphate so that a slight positive meniscus would be formed. A cover slip was placed on top of the tube for 15 min, removed, and placed on a glass slide. The entire area under the cover slip was thoroughly examined by light microscope for the presence of *Giardia* cysts or trophozoites.

3. DNA Extraction

Fresh fecal samples stored at -20˚C and preserved in 70% alcohol were extracted for DNA. Briefly, 200 µl of the fecal suspension was aliquotted, washed three times with distilled water, and centrifuged at 2000 G for 10 min. The DNA was isolated from the pellet using the Qiagen stool mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions.

4. PCR amplification

The SSU-rRNA gene was amplified and yielded 130 bp using primers RH11, RH4 and GiarF and GiarR as previously described [13-14]. All PCR products were separated on a 1.5% agarose gel. The amplified bands were consequently submitted for DNA sequencing compared with known reference sequences using the Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/BLAST).

5. Statistical Analysis

The age of animal was considered one of the risk factors for *Giardia* infection. In addition, herd size and sex were also included in the statistical analysis for predicting their effects on disease distribution. Correlation between the prevalence of *Giardia* infection and factors such as age, sex, province, herd, and herd size was analyzed by using Number Cruncher Statistical System (NCSS) version 2000 (Kaysville, UT) programs and Chi-square (χ^2).

RESULTS

A total of 567 fecal samples from 276 buffalo farms were tested for *Giardia* infection using zinc sulphate floatation technique and nested PCR (nPCR). There were no *Giardia* cysts nor trophozoites in all fecal samples subjected to microscopic examination. Two *G. duodenalis* positive samples (0.4% or 2/567) were found by nPCR based on SSU-rRNA gene and DNA bands of *G. duodenalis* at 130 bp were shown in Figure 2. By the provinces, the highest infection was found in Buri Ram (1.6% or 1/62), followed by Ubon Ratchathani (1.0% or 1/98). For the herd prevalence, only 2 out of 276 herds (overall prevalence of 0.7%) were infected with *G. duodenalis*. Factors including age, sex, herd, herd

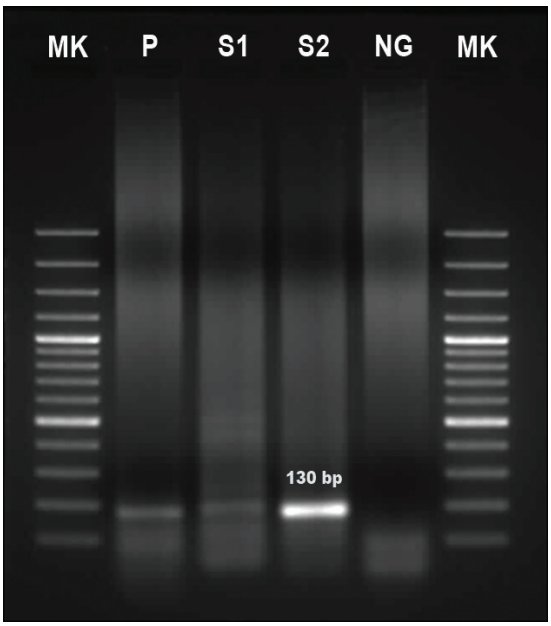

Fig. 2 DNA bands of *G. duodenalis* at approximately 130 bp. (MK = DNA marker 100 bp; P = *Giardia* positive control; S1-S2 = positive samples; NG = negative control)

Table 1 Prevalence of *Giardia* infection in water buffaloes from six provinces of northeast Thailand and its correlation with potential risk factors

Parameter	*Giardia* positive (%)	Statistical parameters
Individual prevalence		
Province		$\chi^2 = 5.48$, df = 5, $p = 0.359$
Buri Ram	1/62 (1.6%)	
Ubon Ratchathani	1/98 (1.0%)	
Roi Et	0/80 (0.0%)	
Si Sa Ket	0/55 (0.0%)	
Surin	0/70 (0.0%)	
Sakon Nakhon	0/202 (0.0%)	
Overall prevalence	2/567 (0.4%)	
Basin		$\chi^2 = 1.11$, df = 1, $p = 0.292$
Sakon Nakhon	0/202 (0.0%)	
Khorat	2/365 (0.5%)	
Age group (year)		$\chi^2 = 1.47$, df = 2, $p = 0.479$
<1	0/4 (0.0%)	
1-5	2/327 (0.6%)	
>5	0/236 (0.0%)	
Sex		$\chi^2 = 2.22$, df = 1, $p = 0.136$
Male	1/78 (1.3%)	
Female	1/489 (0.2%)	
Herd prevalence		
Province		$\chi^2 = 7.20$, df = 5, $p = 0.206$
Buri Ram	1/22 (4.5%)	
Ubon Ratchathani	1/48 (2.1%)	
Roi Et	0/58 (0.0%)	
Si Sa Ket	0/22 (0.0%)	
Surin	0/28 (0.0%)	
Sakon Nakhon	0/98 (0.0%)	
Overall prevalence	2/276 (0.7%)	
Herd size		$\chi^2 = 0.06$, df = 1, $p = 0.806$
≤5 animals	2/268 (0.7%)	
>5 animals	0/8 (0.0%)	

size, and provinces (locations of the fecal samples) were brought to analysis for the correlation with the infection (Table 1). The result showed that higher infections were found in buffaloes aged between 1 and 5 years (0.6%, 2/327), males (1.3%, 1/78), and in small herd size (≤5 buffalo per herd) (0.7%, 2/268). The geographical landscape of northeast areas was divided into Khorat and Sakon

Nakhon Basins [15]. The Khorat basin is located in the South covering Buri Ram, Ubon Ratchathani, Roi Et, Si Sa Ket and Surin Provinces. The Sakon Nakhon Basin is located in the North covering Sakon Nakhon Province. The higher prevalence was found in Khorat Basin (0.5%, 2/365). However the overall results were not statistically significant.

Two PCR positive samples for *G. duodenalis* were sequenced and compared to reference nucleotide sequences from GenBank using BLAST. For assemblage identification, one positive sequence from the buffalo in Buri Ram Province was 100% identical to Assemblage D of *G. duodenalis*. The other sequence from Ubon Ratchathani Province was 100% identical to assemblage E.

DISCUSSIONS

This study is the first report of *Giardia duodenalis* infection in water buffaloes in Northeast Thailand. The overall prevalence of *Giardia* spp. infection in water buffaloes in this study was 0.4% (2/567) compared to 0.7% (2/297) *Giardia* infection in river buffaloes in Sri Lanka [16]. In addition, the prevalence in this study was relatively low when compared to 13% (62/476) and 14% (8/57) *Giardia* infection in water buffaloes in Australia [10] and in Italy [11], respectively. For the other studies in Thailand, the prevalence of *Giardia* infection have been reported in animals including dairy cattle (1%, 4/400) [17] and dogs (70.7%, 162/229) [18]. Nevertheless, the prevalence of giardiasis in Thai people in the North was ranged from 0.9-14.9% in the North [19,20], 0.4-37.7% in the Central [21-23], 1.2-2.2% in the Northeast [22,25], and 4.1-13.1% in the West [23,24].

A diversity of genetic markers for genotyping *G. duodenalis* was based on small subunit ribosomal RNA (SSU-rRNA), β-giardin (*bg*), glutamate dehydrogenase (*gdh*), elongation factor 1-alpha (*ef1*α), triose phosphate isomerase (*tpi*), GLORF-C4 (*C4*), and inter genomic rRNA spacer region (*IGS*) [26]. In this region, the SSU-rRNA sequence is more conserved and sensitive for the detection of different assemblages; however, intra-assemblage variation of this gene is limited. The *bg*, *gdh*, and *tpi* genes have shown more intra-assemblage variation that were used to discriminate assemblages A (AI and AII) and B (BIII and BIV) [26]. The SSU-rRNA gene contains the variable at 5′ and 3′ ends that can be used to identify *G. duodenalis* assemblage, so that the specific conserved regions of this gene would be used for identifying *Giardia* species [27]. However, the short sequence of PCR product (140-280 bp) might not be sufficient to differentiate *G. duodenalis* assemblages [27].

G. duodenalis Assemblages A and B are considered potential zoonotic pathogens. Assemblages A and B exist and are transmitted among humans, wildlife, domestic and farm animals [8]. The highest risk of giardiasis transmission might come from pets such as dogs and cats that are close to humans [8]. However, the infected dairy cattle were also an important reservoir for giardiasis in humans [2]. The transmission of zoonotic assemblages from cattle to humans had been associated with water-borne outbreaks in USA [2]. A few studies of molecular characterization of *Giardia* assemblages in water buffaloes have been reported as Assemblages E and A [10,11,16,28]. In Italy and Sri Lanka, Assemblage E found in water buffaloes has been reported more than Assemblage A [11,16] while water buffaloes in Australia were infected with only Assemblage A [10]. *G. duodenalis* Assemblage E was also found in cattle, sheep, and pigs and this assemblage was predominantly distributed among cattle in North America, Europe and Australia [27]. In this study, *G. duodenalis* infection of buffaloes in Thailand, based on SSU-rRNA gene, were identified as Assemblages D and E.

Giardia duodenalis infections found in young animals (less than 1 year) particularly animals between 2–10 weeks are significantly high [29-30]. For Thai community in the rural area, buffaloes were roaming in the public pasture such as rice and corn fields and these areas are polluted by the animals' feces leading to the transmission of *Giardia* to humans under poor sanitary and improper hygiene. During the agricultural practice in the rural area, humans might also defecate in the field; this will complete the cycle

of *Giardia* transmission. *Giardia* cysts can survive for months in surface water and in soil [27] so that people in nearby environment might be at high risks of ingesting contaminated *Giardia* cysts in their water or food.

ACKNOWLEDGMENTS

This research is supported by Center for Agricultural Biotechnology (CAB), Kasetsart University, Kamphaengsan Campus and Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Commission on Higher Education, Ministry of Education. (AG-BIO/PERDO-CHE).

REFERENCES

1. Thompson RC, Monis PT. Variation in *Giardia*: implications for taxonomy and epidemiology Adv Parasitol. 2004;58:69-137.
2. Ryan U, Caccio SM. Zoonotic potential of *Giardia*. Int J Parasitol. 2013;43:943-56.
3. Koehler AV, Jex AR, Haydon SR, Stevens MA, Gasser RB. *Giardia*/giardiasis - A perspective on diagnostic and analytical tools. Biotechnol Adv. 2014;32:280-9.
4. WHO. The World Health Report 1996: fighting disease, fostering development. Geneva: World Health Organization; 1996.
5. Dixon B, Parrington L, Cook A, Pintar K, Pollari F, Kelton D, *et al*. The potential for zoonotic transmission of *Giardia duodenalis* and *Cryptosporidium* spp. from beef and dairy cattle in Ontario, Canada. Vet Parasitol. 2011;175:20-6.
6. Coklin T, Farber J, Parrington L, Dixon B. Prevalence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in dairy cattle in Ontario, Canada. Vet Parasitol. 2007;150:297-305.
7. Trout JM, Santín M, Greiner EC, Fayer R. Prevalence and genotypes of *Giardia duodenalis* in 1-2 year old dairy cattle. Vet Parasitol. 2006;140:217-22.
8. Thompson RC. The zoonotic significance and molecular epidemiology of *Giardia* and giardiasis. Vet Parasitol. 2004;126:15-35.
9. Budu-Amoako E, Greenwood SJ, Dixon BR, Barkema HW, McClure JT. Foodborne illness associated with *Cryptosporidium* and *Giardia* from livestock. J Food Prot. 2011;74:1944-55.
10. Abeywardena H, Jex AR, von Samson-Himmelstjerna G, Haydon SR, Stevens MA, Gasser RB. First molecular characterization of *Cryptosporidium* and *Giardia* from *Bubalus bubalis* (water buffalo) in Victoria, Australia. Infect Genet Evol. 2013;20:96-102.
11. Cacciò SM, Rinaldi L, Cringoli G, Condoleo R, Pozio E. Molecular identification of *Cryptosporidium parvum* and *Giardia duodenalis* in the Italian water buffalo (*Bubalus bubalis*). Vet Parasitol. 2007;150:146-9.
12. Perera BM. Reproductive cycles of buffalo. Anim Reprod Sci. 2011;124:194-9.
13. Hopkins RM, Meloni BP, Groth DM, Wetherall JD, Reynoldson JA, Thompson RC. Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolations recovered from humans and dogs living in the same locality. J Parasitol. 1997;83:44-51.
14. Sahagún J, Clavel A, Goñi P, Seral C, Llorente MT, Castillo FJ, *et al*. Correlation between the presence of symptoms and the *Giardia duodenalis* genotype. Eur J Clin Microbiol Infect Dis. 2008;27:81-3.
15. Potapon M. GMS regional trade in livestock: animal flows and disease risk. Food and Agriculture Organization of The United Nations Regional Office for Asia and The Pacific, Bangkok, 2013 [updated 2013 June 8; cited 2014 April 1]. Available from: http://cdn.aphca.org/dmdocuments/PAP_13_GMS%20Lstk%20Flows_Potapon_130603.pdf
16. Abeywardena H, Jex AR, Koehler AV, Rajapakse RJ, Udayawarna K, Haydon SR, *et al*. First molecular characterization of *Cryptosporidium* and *Giardia* from bovines (*Bos taurus* and *Bubalus bubalis*) in Sri Lanka: unexpected absence of *C. parvum* from pre-weaned calves. Parasit Vectors. 2014;7:1-10.
17. Inpankaew T, Traub R, Thadtapong N,

Pinyopanuwat N, Chimnoi W, Jittapalapong S. Prevalence of giardiasis in dairy cattle in northeastern part of Thailand. Proceedings of the 48th Kasetsart University Annual Conference; 2010 March 3-5; Bangkok, Thailand.

18. Traub RJ, Inpankaew T, Reid SA, Sutthikornchai C, Sukthana Y, Robertson ID, *et al*. Transmission cycles of *Giardia duodenalis* in dogs and humans in temple communities in Bangkok-A critical evaluation of its prevalence using three diagnostic tests in the field in the absence of a gold standard. Acta Trop. 2009;111:125-32.
19. Waree P, Polseela P, Pannarunothai S, Pipitgool V. The present situation of paragonimiasis in endemic area in Phitsanulok Province. Southeast Asian J Trop Med Public Health. 2001;32:51-4.
20. Piangjai S, Sukontason K, Sukontason KL. Intestinal parasitic infections in hill-tribe schoolchildren in Chiang Mai, Northern Thailand. Southeast Asian J Trop Med Public Health. 2003;34:90-3.
21. Saksirisampant W, Nuchprayoon S, Wiwanitkit V, Yenthakam S, Ampavasiri A. Intestinal parasitic infestations among children in an orphanage in Pathum Thani province. J Med Assoc Thai. 2003;86 (Suppl 2):263-70.
22. 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 (Suppl 4): 21-5.
23. Tungtrongchitr A, Sookrung N, Indrawattana N, Kwangsi S, Ongrotchanakun J, Chaicumpa W. *Giardia intestinalis* in Thailand: identification of genotypes. J Health Popul Nutr. 2010;28:42-52.
24. Pothipak N, Srivilairit S, Pengruksa C, Faithong S, Haohan O, Chalermrut K, *et al*. Health Status: malaria, anemia and intestinal parasitic infections on the Thai-Myanmar Border. J Trop Med Parasitol. 2005;28:26-30.
25. Tungtrongchitr A, Chiworaporn C, Praewanich R, Radomyos P, Boitano JJ. The potential usefulness of the modified Kato thick smear technique in the detection of intestinal sarcocystosis during field surveys. Southeast Asian J Trop Med Public Health. 2007;38:232-8.
26. Cacciò SM1, Ryan U. Molecular epidemiology of giardiasis. Mol Biochem Parasitol. 2008;160:75-80.
27. Feng Y, Xiao L. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microbiol Rev. 2011;24: 110-40.
28. Cacciò SM, Beck R, Lalle M, Marinculic A, Pozio E. Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. Int J Parasitol. 2008;38:1523-31.
29. Xiao L, Fayer R. Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. Int J Parasitol. 2008;38:1239-55.
30. Olson ME, O'Handley RM, Ralston BJ, McAllister TA, Thompson RC. Update on *Cryptosporidium* and *Giardia* infections in cattle. Trends Parasitol. 2004;20:185-91.