FULL PAPER
Parasitology

Prevalence, genotyping and risk factors of *Giardia duodenalis* from dogs in Vietnam

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ABSTRACT. This study was carried out to determine the prevalence, genotypes/assemblages and possible risk factors associated with *Giardia duodenalis* infection in dogs in central Vietnam. A total of 209 dog fecal samples, randomly collected from private owned dogs (n=105) and dogs from stores (n=104), were examined for *Giardia* cysts by microscopy. Positive samples were genotyped by PCR-sequence analysis of β-giardin and triosephosphate isomerase genes markers. Risk factors were studied using a structured questionnaire and collected data were analyzed by univariate and multivariate logistic regression analyses. Results indicated that the overall infection rate was 8.6% (18/209) with the detected parasites were belonging to the non-zoonotic assemblages C and D. Age, gender and origin of animals were the main risk factors associated with *G. duodenalis* infection in dogs under study. Occurrence of infection was more likely in young animals compared to old ones and in females compared to males. Dogs originated from stores were more prone to *Giardia* infection compared to private owned counterparts.

KEY WORDS: dog, genotyping, *Giardia*, risk assessment

Giardia duodenalis (syn. *Giardia intestinalis*, *Giardia lamblia*) is a ubiquitous enteric protozoan parasite that infects a wide range of vertebrate hosts [3]. The infection is transmitted by the fecal-oral route via ingestion of the infective cysts through contaminated food and/or water [3]. The course of the disease is highly variable, ranging from asymptomatic infection to acute or chronic illness with diarrhea [10]. In human, *G. duodenalis* is frequently associated with outbreaks and sporadic cases [20], with estimation of 500,000 annual infections worldwide, mostly in children in Asia, Africa and Latin America [26].

Giardiasis is common in dogs, with infection rate ranging from 5–70% [3]. Of the eight genotypes/assemblages (A to H), that have been recognized within *G. duodenalis* complex species, assemblages C and D are canine-specific, however assemblages A, B and E were also reported [9, 20].

In Vietnam, molecular studies were focused on *G. duodenalis* in cattle [4, 13], with obvious scarcity of data from other hosts. Vietnamese people are usually raising dogs for the purpose of house-keeping and as companion animals. Although dogs in such situations are in close contact with humans, no data are available on the occurrence and identity of *Giardia* parasites in such dogs. Therefore, the present study was designed to investigate the prevalence and risk factors of *Giardia* infection in dogs in Vietnam, and molecularly characterize the obtained isolates up to assemblage level, thereby to assess the zoonotic potential of the detected parasites.

MATERIALS AND METHODS

Collection of samples

During the period from October 2016 to March 2017, a total of 209 fecal samples were collected from dogs in DacLac (n=111) and KhanhHoa (n=98) provinces, including private owned dogs (n=105) and dogs in stores (n=104). The dog population was divided into three age groups: <12 months (n=73), 12–36 months (n=92) and >36 months (n=44). For private owned dogs, animals were living indoors and had the chance of controlled roaming outside once or twice a day. For dogs from stores, animals were kept in metal kennels with supplementary food. In fact, dogs in stores are not always kept in individual cages, each metal cage usually contains from 1–2 heads, depending on convenience of the cage. Sampled dogs were Vietnamese native breed, native cross breed

Table 1. Univariate analysis of factors predicted to be associated with the presence of *Giardia duodenalis* in dogs in central Vietnam

Factor	*n*	No positive (%)	*P*-value
Location (province)			0.441
KhanhHoa	98	10 (10.2)	
DacLac	111	8 (7.2)	
Age (months)			0.019
<12	73	12 (16.4)	
12–36	92	4 (4.3)	
>36	44	2 (4.5)	
Gender of animals			0.038
Male	107	5 (4.7)	
Female	102	13 (12.7)	
Breed of animals			0.214
Japanese crossed	12	0 (0)	
Germanic crossed	5	0 (0)	
Vietnamese native pure	135	16 (11.9)	
Mixed	57	2 (3.5)	
Clinical signs (Diarrhea)			0.081
Yes	31	0 (0)	
No	178	18 (10.1)	
Sterilized status			0.39
Yes	20	3 (15.0)	
No	189	15 (7.9)	
Origins of animals			0.046
Private owned	105	5 (4.8)	
Stores	104	13 (12.5)	
Environmental conditions			0.293
Urban area	150	11 (7.3)	
Suburban area	59	7 (11.9)	

with Japanese and native cross breed with Germanic dogs along with mixed unknown breed(s) (Table 1). Fecal samples were collected from the floor immediately after defecation in the morning, placed in individual plastic bags, kept at 4–8°C and processed within 2–4 days after collection.

Individual animal information was obtained from the owner of the animal and the master of the store, through a designed questionnaire. The questionnaire addressed the following points: the age and clinical status of the animals, the gender, breed of dogs and sterilized status, the origin of the animals and environmental conditions of dogs. The protocol of the present study was approved by the Scientific Committee of Central Vietnam Veterinary Institute. No experimentation was done on the assigned animals.

Primary screening of all fecal samples, using approximately 1.5 g faeces per sample, was performed by zinc-sulfate flotation method [8], combined with iodine staining and examined by light microscopy according to the criteria described by Nguyen *et al*. [13].

DNA extraction and molecular analysis

Total DNA was extracted from all microscopically positive samples using a FavorPrep Stool DNA Isolation Mini Kit (Favorgen Biotech Corporation, Changzhi, Ping-Tung, Taiwan), according to the manufacturer's instructions. The eluted DNA was dissolved in 30 μl of ultra-pure water and stored at −20°C.

The DNA fragments of the β-giardin (*bg*) (−510 bp) and triosephosphate isomerase (*tpi*) (−530 bp) genes were amplified by polymerase chain reaction (PCR), utilizing the primer sets and protocols described by Lalle *et al*. [7] and Sulaiman *et al*. [18], respectively. Negative (without template) controls were included in each PCR batch.

The PCR products were purified using ExonucleaseI/Shrimp Alkaline Phosphatase (Exo-SAP-ITTM) (USB Corporation, Cleveland, OH, U.S.A.). Purified products were directly sequenced in a 20 μl reaction volume using the Big Dye Terminator Cycle sequencing kit version 3.1 on an automated sequencer (Applied Biosystems 3130*xl*, genetic analyzer), (Life Technologies Japan Ltd., Tokyo, Japan). The accuracy of data was confirmed by two-directional sequencing. The obtained sequences were blasted against the GenBank database to determine *G. duodenalis* genotypes/assemblages, covering a region of approximately 475 and 490 bp for bg and tpi genes, respectively. Phylogenetic trees based on both genes were constructed with the neighbor-joining algorithm using the software package MEGA, version 5.2. [19]. The statistical confidence of branching patterns was evaluated by a bootstrap test with 1,000 replications. Representative nucleotide sequences were deposited in the GenBank under the accession numbers LC 316658, LC 316659 for bg gene and LC 316660 for tpi gene.

Table 2. *Giardia duodenalis* assemblages identified by sequencing of the *bg* and *tpi* genes in isolates from dogs in Vietnam

No	Isolate ID	Age group (months)	Assemblage at *bg* [a] locus	Assemblage at *tpi* [b] locus
1	KH1	<12	C	C
2	KH2	<12	C	C
3	KH3	<12	D	C
4	KH4	<12	C	C
5	KH5	<12	D	C
6	KH6	<12	C	C
7	KH7	<12	C	C
8	KH8	<12	C	C
9	KH9	12–36	D	C
10	KH10	>36	D	N/A [c]
11	DL1	<12	C	C
12	DL2	<12	C	C
13	DL3	<12	C	C
14	DL4	12–36	D	N/A [c]
15	DL5	12–36	C	C

a) *bg*=β-giardin, b) *tpi*=triosephosphate isomerase, c) N/A=No Amplification.

Statistical analyses

Statistical analyses were performed using the STATA, version 12.0 software (StataCorp LP, Lakeway Drive, College Station, TX, U.S.A.). Univariate logistic regression analysis was performed to determine the association between *Giardia* status and putative risk factors using Fisher's exact test or χ^2 test. Only variables significant in the univariate analysis were further retained in the multivariate logistic regression analysis, which was undertaken to estimate the odds ratios for all such variables. The Akaike's Information Criterion was used as the calibrating parameter to select the final model. In both the univariate and multivariate analyses, associations were considered to be significant at $P<0.05$.

RESULTS

Occurrence and identity of Giardia parasites

Out of the 209 fecal samples examined by microscopy, 18 (8.6%) samples showed the presence of *Giardia* cysts.

Molecularly, the expected PCR products of the *bg* and *tpi* gene fragments were obtained from 15/18 and 13/18 samples, respectively (Table 2). The *bg* gene sequences of 10 isolates (represented by sequence LC316658) were identical and showed 100% homology with those (JF422720 and AY545646) of *G. duodenalis* assemblage C from dogs. In addition, five sequences from 5 isolates (represented by sequence LC316659) showed 100% homology with those of *G. duodenalis* assemblage D (KJ027423 and AY545647) derived from dogs. The nucleotide sequences of the *tpi* gene of all 13 isolates were identical (represented by sequence LC316660) and showed 99% homology with those of *G. duodenalis* assemblage C (KY979493, HG970114 and KX014796). The phylogenetic trees based on the *bg* and *tpi* genes are shown in Figs. 1 and 2, respectively.

Risk factor analysis

The result of the univariate logistic regression analysis for risk factors is presented in Table 1. Out of 8 predicted factors, three factors including age, gender and origins showed significant association with *Giardia* infection ($P<0.05$). In contrast, factors including location, breed, clinical status, sterilized status and environmental conditions were not significantly associated with the infection.

In multivariate logistic regression analysis (Table 3), age, gender and origins of the animals were confirmed to be significantly risk factors associated with infection status. Risk factor of *Giardia* infection decreased with the advance in animal age. Based on the analysis, dogs under 12 months were 4.01 (95% CI: 1.19, 13.41) times more likely to be infected with *Giardia* than those between 12–36 and >36 months in age. Female dogs were 6.7 (95% CI: 1.31, 34.7) times more prone to infection than male ones. Dogs originated from stores were 3.22 (95% CI: 1.05, 9.88) times at risk of infection than private owned dogs.

DISCUSSION

In the present study, the prevalence and identity of *G. duodenalis* in dogs in Vietnam was investigated. *G. duodenalis* cysts were detected in 18 of 209 (8.6%) dog fecal samples examined by microscopy. This figure is similar to that previously reported in dogs from China (8.6%) [8] and Thailand (7.9%) [22], using the same examination method. However, it is lower than that reported in other areas using PCR or ELISA techniques including China (11–16%) [8, 25], Trinidad and Tobago (25%) [11], U.S.A. (15%) [12], U.K. (21%) [24] and Italy (57.9%) [17]. There are several factors might contribute to differences in infection rates such as the
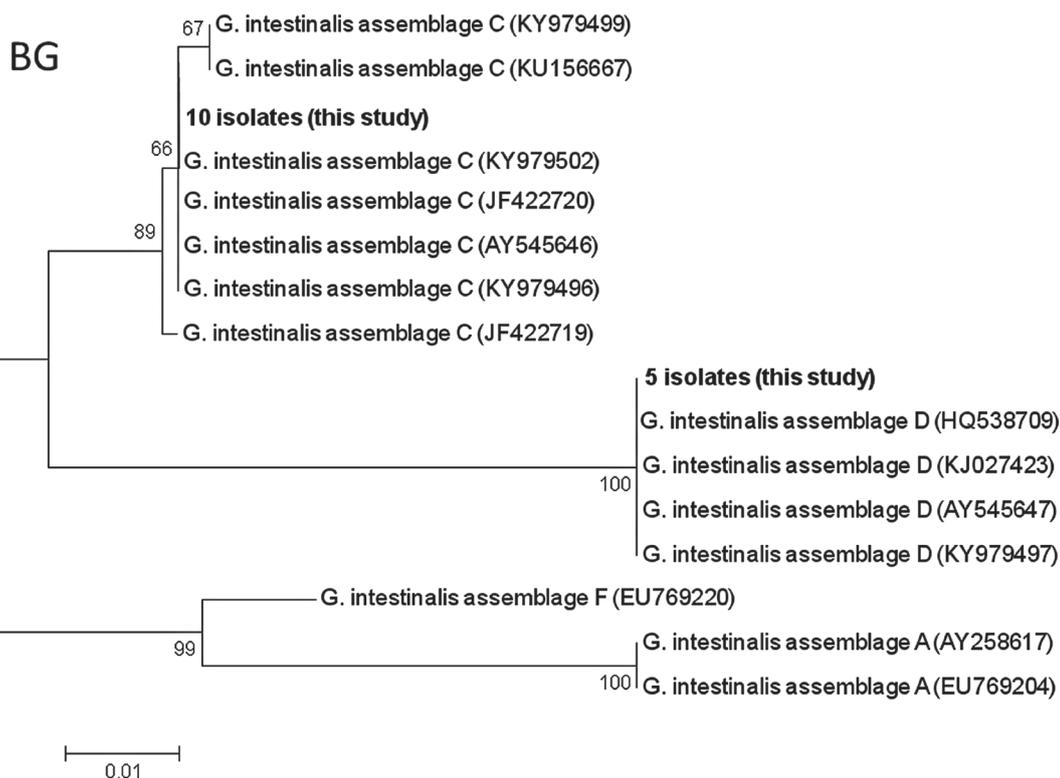
Fig. 1. Phylogenetic relationships of *Giardia* isolates in dogs using partial β–giardin sequences. Accession numbers are shown in parentheses.
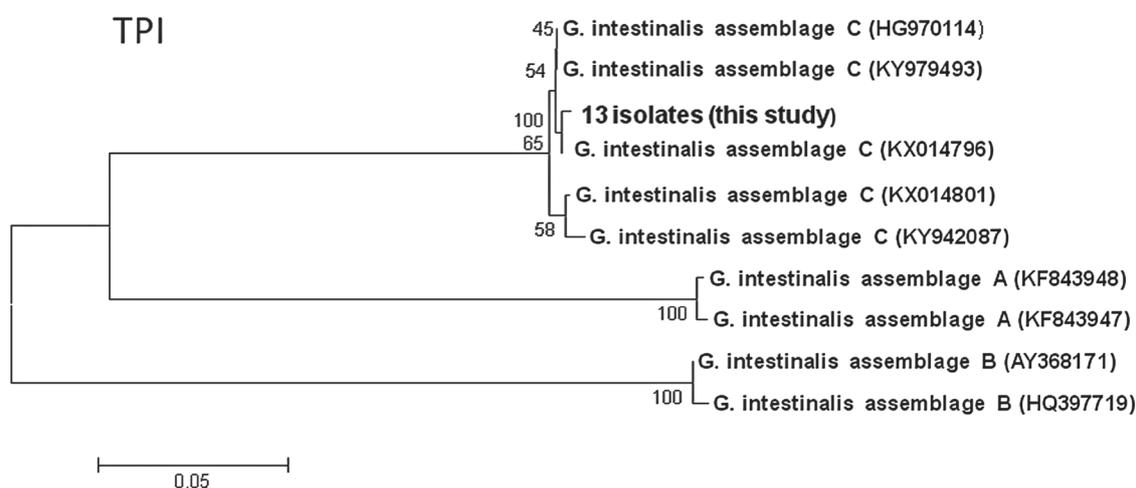

Fig. 2. Phylogenetic relationships of *Giardia* isolates in dogs using partial triosephosphate isomerase sequences. Accession numbers are shown in parentheses.

age of target animal population, the applied diagnostic method, the life styles of the animals and sampling schedule [11].

Statistical analysis indicated that the prevalence of *Giardia* significantly decreased with increasing of the age of animals, they were detected in dogs aged >12 months in a limited number (6/136). In concordance, previous studies [8, 15, 25] showed a strong correlation between the age and presence of *Giardia* cysts in stool samples. The same tendency was also observed in *Giardia* infected cats by Itoh *et al*. [6] in Japan. This might be linked to the possibility of frequent exposure of old animals to infection which might provide some sort of protection [15]. Thus, separating the dogs <12 months of age from older animals might be an effective way to reduce exposure to *Giardia* parasite within the dogs.

Results reported herein showed that infection of dogs with *Giardia* appears to vary significantly between dogs from two different origins. In agreement with results of previous reports [11, 23], dogs from stores were at higher risk of infection compared to private owned ones. In fact, dogs originated from stores are usually kept in metal kennels, which might create less hygienic conditions

Table 3. Multivariate analysis to determine factors associated with the presence of *Giardia duodenalis* in dogs in central Vietnam

Variable	Odds ratio	95% CI	*P*-value
Age (months)			0.021 [b]
<12	4.01	1.19−13.41	0.024
12–36	1 [a]		
>36	1.23	0.20−7.5	0.819
Gender			0.016 [b]
Male	1 [a]		
Female	6.7	1.31−34.7	0.022
Origins of animals			0.045 [b]
Private owned	1 [a]		
Stores	3.22	1.05−9.88	0.041

a) Reference group, b) Overall *P*-value of variable.

and stressful situation on the animals, rendering them to be more vulnerable to the infection. In addition, the dogs in stores are not always bred in individual kennels, therefore cross infection easily occur in stores by contact with infected individuals or handling by staff. Also, a significant variation by gender group was found, however, the findings that female dogs were more prone to the infection compared to male counterparts were unexpected, and require further investigations using large sample size. In contrast, breed of dogs did not influence the prevalence of *Giardia,* although they showed a certain impact on the cyst shedding in other studies [14, 24].

In contrast to previous findings [8], the present study could not find clear association between diarrhea and infection. As mentioned by Geurden *et al.* [5], *G. duodenalis* causes chronic yet intermittent diarrhea in infected animals, making it difficult to find a correlation between this parameter and infection through a one sampling study design. Of note, several causes including nutritional and microbial factors may be implicated in occurrence of diarrhea not on the focus of the present study, and needs further investigations.

Molecular analysis in the *bg* and *tpi* genes revealed presence of the *G. duodenalis* assemblage C and D in dogs examined. Based on *tpi* locus, all amplified PCR products were determined as assemblage C by direct sequence analysis. However, sequence results based on *bg* locus, indicated that 10 samples were determined as assemblages C, and 5 samples as assemblages D. The phenomenon of equivocal assignation at the assemblage level by different genes has been observed in several genotyping studies [1, 16, 21], which was attributed to recombination process or presence of concurrent infection with more than one assemblage [16]. The result in our study was in accordance with the conclusion of dominance of assemblage C and D in dogs worldwide [3], although assemblage A and to a lesser extent, assemblage B were also detected in few surveys [2, 12].

In conclusion, this study presented a survey of risk factors and molecular characterizations of *Giardia* parasite in dogs in Vietnam. It showed that age, gender and origins of the animals were significantly associated with *Giardia* cyst shedding. Prevalence decreased with increasing age and increased in female dogs, whereas animals originated from stores were more likely to be infected with *Giardia* than private owned dogs. Since the assemblages C and D detected in the present study have no known zoonotic potential, risk of infection to humans is minimal. However, further studies on prevalence, genotyping and risk analysis of *Giardia* in humans and other livestock are warranted in order to obtain consolidated conclusion of giardiasis in Vietnam.

REFERENCES

1. Cacciò, S. M., Beck, R., Lalle, M., Marinculic, A. and Pozio, E. 2008. Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. *Int. J. Parasitol.* **38**: 1523–1531. [Medline] [CrossRef]
2. Fava, N. M., Soares, R. M., Scalia, L. A., Cunha, M. J., Faria, E. S. and Cury, M. C. 2016. Molecular typing of canine *Giardia duodenalis* isolates from Minas Gerais, Brazil. *Exp. Parasitol.* **161**: 1–5. [Medline] [CrossRef]
3. Feng, Y. and Xiao, L. 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clin. Microbiol. Rev.* **24**: 110–140. [Medline] [CrossRef]
4. Geurden, T., Somers, R., Thanh, N. T., Vien, L. V., Nga, V. T., Giang, H. H., Dorny, P., Giao, H. K. and Vercruysse, J. 2008. Parasitic infections in dairy cattle around Hanoi, northern Vietnam. *Vet. Parasitol.* **153**: 384–388. [Medline] [CrossRef]
5. Geurden, T., Vanderstichel, R., Pohle, H., Ehsan, A., von Samson-Himmelstjerna, G., Morgan, E. R., Camuset, P., Capelli, G., Vercruysse, J. and Claerebout, E. 2012. A multicentre prevalence study in Europe on *Giardia duodenalis* in calves, with molecular identification and risk factor analysis. *Vet. Parasitol.* **190**: 383–390. [Medline] [CrossRef]
6. Itoh, N., Muraoka, N., Kawamata, J., Aoki, M. and Itagaki, T. 2006. Prevalence of *Giardia intestinalis* infection in household cats of Tohoku district in Japan. *J. Vet. Med. Sci.* **68**: 161–163. [Medline] [CrossRef]
7. Lalle, M., Pozio, E., Capelli, G., Bruschi, F., Crotti, D. and Cacciò, S. M. 2005. Genetic heterogeneity at the beta-giardin locus among human and

animal isolates of *Giardiaduodenalis* and identification of potentially zoonotic subgenotypes. *Int. J. Parasitol.* **35**: 207–213. [Medline] [CrossRef]

8. Li, J., Zhang, P., Wang, P., Alsarakibi, M., Zhu, H., Liu, Y., Meng, X., Li, J., Guo, J. and Li, G. 2012. Genotype identification and prevalence of *Giardia duodenalis* in pet dogs of Guangzhou, Southern China. *Vet. Parasitol.* **188**: 368–371. [Medline] [CrossRef]

9. Li, W., Li, Y., Song, M., Lu, Y., Yang, J., Tao, W., Jiang, Y., Wan, Q., Zhang, S. and Xiao, L. 2015. Prevalence and genetic characteristics of Cryptosporidium, Enterocytozoon bieneusi and Giardia duodenalis in cats and dogs in Heilongjiang province, China. *Vet. Parasitol.* **208**: 125–134. [Medline] [CrossRef]

10. Liu, A., Zhang, X., Zhang, L., Wang, R., Li, X., Shu, J., Zhang, X., Shen, Y., Zhang, W. and Ling, H. 2012. Occurrence of bovine giardiasis and endemic genetic characterization of *Giardia duodenalis* isolates in Heilongjiang Province, in the Northeast of China. *Parasitol. Res.* **111**: 655–661. [Medline] [CrossRef]

11. Mark-Carew, M. P., Adesiyun, A. A., Basu, A., Georges, K. A., Pierre, T., Tilitz, S., Wade, S. E. and Mohammed, H. O. 2013. Characterization of *Giardia duodenalis* infections in dogs in Trinidad and Tobago. *Vet. Parasitol.* **196**: 199–202. [Medline] [CrossRef]

12. Munoz, J. and Mayer, D. C. 2016. *Toxoplasma gondii* and *Giardia duodenalis* infections in domestic dogs in New York City public parks. *Vet. J.* **211**: 97–99. [Medline] [CrossRef]

13. Nguyen, S. T., Fukuda, Y., Nguyen, D. T., Tada, C. and Nakai, Y. 2016. Prevalence and first genotyping of *Giardia duodenalis* in beef calves in Vietnam. *Trop. Anim. Health Prod.* **48**: 837–841. [Medline] [CrossRef]

14. Pallant, L., Barutzki, D., Schaper, R. and Thompson, R. C. 2015. The epidemiology of infections with *Giardia* species and genotypes in well cared for dogs and cats in Germany. *Parasit. Vectors* **8**: 2. [Medline] [CrossRef]

15. Pipia, A. P., Varcasia, A., Tamponi, C., Sanna, G., Soda, M., Paoletti, B., Traversa, D. and Scala, A. 2014. Canine giardiosis in Sardinia Island, Italy: prevalence, molecular characterization, and risk factors. *J. Infect. Dev. Ctries.* **8**: 655–660. [Medline] [CrossRef]

16. Santín, M., Cortés Vecino, J. A. and Fayer, R. 2013. A large scale molecular study of *Giardia duodenalis* in horses from Colombia. *Vet. Parasitol.* **196**: 31–36. [Medline] [CrossRef]

17. Simonato, G., Frangipane di Regalbono, A., Cassini, R., Traversa, D., Beraldo, P., Tessarin, C. and Pietrobelli, M. 2015. Copromicroscopic and molecular investigations on intestinal parasites in kenneled dogs. *Parasitol. Res.* **114**: 1963–1970. [Medline] [CrossRef]

18. Sulaiman, I. M., Fayer, R., Bern, C., Gilman, R. H., Trout, J. M., Schantz, P. M., Das, P., Lal, A. A. and Xiao, L. 2003. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. *Emerg. Infect. Dis.* **9**: 1444–1452. [Medline] [CrossRef]

19. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**: 2731–2739. [Medline] [CrossRef]

20. Thompson, R. C., Palmer, C. S. and O'Handley, R. 2008. The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. *Vet. J.* **177**: 18–25. [Medline] [CrossRef]

21. Traub, R. J., Monis, P. T., Robertson, I., Irwin, P., Mencke, N. and Thompson, R. C. 2004. Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. *Parasitology* **128**: 253–262. [Medline] [CrossRef]

22. Traub, R. J., Inpankaew, T., Reid, S. A., Sutthikornchai, C., Sukthana, Y., Robertson, I. D. and Thompson, R. C. 2009. 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.* **111**: 125–132. [Medline] [CrossRef]

23. Uehlinger, F. D., Greenwood, S. J., McClure, J. T., Conboy, G., O'Handley, R. and Barkema, H. W. 2013. Zoonotic potential of *Giardia duodenalis* and *Cryptosporidium* spp. and prevalence of intestinal parasites in young dogs from different populations on Prince Edward Island, Canada. *Vet. Parasitol.* **196**: 509–514. [Medline] [CrossRef]

24. Upjohn, M., Cobb, C., Monger, J., Geurden, T., Claerebout, E. and Fox, M. 2010. Prevalence, molecular typing and risk factor analysis for Giardia duodenalis infections in dogs in a central London rescue shelter. *Vet. Parasitol.* **172**: 341–346. [Medline] [CrossRef]

25. Yang, D., Zhang, Q., Zhang, L., Dong, H., Jing, Z., Li, Z. and Liu, J. 2015. Prevalence and risk factors of Giardia duodenalis in dogs from China. *Int. J. Environ. Health Res.* **25**: 207–213. [Medline] [CrossRef]

26. Yason, J. A. and Rivera, W. L. 2007. Genotyping of *Giardia duodenalis* isolates among residents of slum area in Manila, Philippines. *Parasitol. Res.* **101**: 681–687. [Medline] [CrossRef]