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▣ BRIEF COMMUNICATION

Molecular Detection of *Giardia intestinalis* from Stray Dogs in Animal Shelters of Gyeongsangbuk-do (Province) and Daejeon, Korea

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Abstract: *Giardia* is a major public health concern and considered as reemerging in industrialized countries. The present study investigated the prevalence of giardiosis in 202 sheltered dogs using PCR. The infection rate was 33.2% (67/202); Gyeongsangbuk-do and Daejeon showed 25.7% (39/152, $P<0.0001$) and 56% (28/50), respectively. The prevalence of infected female dogs (46.7%, $P<0.001$) was higher than in male dogs (21.8%). A higher prevalence (43.5%, $P<0.0001$) was observed in mixed breed dogs than purebred (14.1%). Although most of the fecal samples collected were from dogs of ≥1 year of age which showed only 27.4% positive rate, 61.8% ($P<0.001$) of the total samples collected from young animals (<1 year of age) were positive for *G. intestinalis*. A significantly higher prevalence in symptomatic dogs (60.8%, $P<0.0001$) was observed than in asymptomatic dogs (23.8%). Furthermore, the analysis of nucleotide sequences of the samples revealed that *G. intestinalis* Assemblages A and C were found in the feces of dogs from Gyeongsangbuk-do and Daejeon. Since *G. intestinalis* Assemblage A has been known to infect humans, our results suggest that dogs can act as an important reservoir of giardiosis in Korea. Hence, hygienic management should be given to prevent possible transmission to humans.

Key words: *Giardia intestinalis*, dog, PCR

Giardia is a major public health concern of water utilities worldwide, and is considered as reemerging in industrialized countries due to its role in diarrheal, and in water and foodborne outbreaks [1-3]. It is an aerotolerant enteric protozoan parasite which can cause giardiosis, infecting a wide range of vertebrate hosts including humans characterized by diarrhea, bloating, abdominal cramps, weight loss, and malabsorption [4,5]. It is one of the most common enteric parasites of companion animals and livestock, and has been observed as one of the most frequently observed parasites infecting domestic dogs [2,6]. A recent study reported that during the period of 2004-2010, 70 out of 199 published protozoal disease outbreaks were caused by *Giardia* [7].

G. intestinalis, also known as *G. lamblia* and *G. duodenalis*, is the only species that has been recovered from both humans and dogs, hence regarded as potentially zoonotic [8]. Furthermore, it has several genotypes such as assemblages C and D, which are the most commonly found in dogs, and assemblages A and B, which are the most commonly found in humans and have also been isolated in dogs, suggesting a potential source of infection to humans [9].

Microscopic fecal examination has been the routine detection method of *G. intestinalis* in dogs; however, this method is considered limited particularly in the presence of concurrent infections with multiple parasite species and insufficient to detect the parasites and many cases of *Giardia* infections go undetected [10]. Molecular diagnostic tools are thought to provide higher sensitivity and specificity compared to either microscopic or immunological assays and have been used recently in characterizing the epidemiology of human giardiosis, and in assessing the taxonomy, zoonotic potential, and transmission of giardiosis in humans and animals [2,5,11]. PCR assay is a sensitive and specific method for detecting microorganisms by amplifying the target nucleic acids, and molecular detection methods based on this assay have been shown to be highly rapid, sensitive, and specific for detection of *G. intesti-*

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nalis cysts [12]. Consequently, the present study examined 202 canine fecal samples to determine the presence of giardiosis in dogs from animal shelters in Korea using PCR.

A total of 202 canine fecal samples collected from each animal shelter in Gyeongsangbuk-do (Province) (n=152) and Daejeon city (n=50) from February 2013 to February 2015 were used in the study. All cases were grouped according to gender, breed (mixed/pure), age group (≥1 year/<1 year), and health status (symptomatic/asymptomatic) (Table 1). Health status was based on main clinical manifestations of giardiosis which can range from asymptomatic (without diarrhea), to acute or chronic diarrheal disease (symptomatic) [13]. Moreover, the positive genomic DNA of *G. intestinalis* (ATCC no. 30957) was kindly provided by Dr. S. E. Lee in Division of Malaria and Parasite Diseases, Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Korea. Total genomic DNA extraction of each fecal sample was performed using the QIAamp DNA Stool Mini Kit in accordance with manufacturer's instruction (Qiagen, Hilden, Germany).

A set of primers to amplify a 384 bp fragment of *β-giardin* gene of *G. intestinalis* was amplified using the forward primer (5′ CAT AAC GAC GCC ATC GCG GCT CTC AGG AA 3′) and the reverse primer (5′ GAG GCC GCC CTG GAT CTT CGA GAC GAC 3′) as previously described [14]. The primers were designed based from the complete *β-giardin* gene of the Portland-1 isolate (GenBank no. M36728). The amplification of PCR products was performed in a MyGenie96 Thermal Block thermal cycler (Bioneer, Seoul, Korea), resolved using 1.5% Tris-acetate-EDTA (TAE) in an electrophoresis chamber containing 0.5x TAE buffer, run at 100 V for 25 min or until the dye indicator reached the target lane, stained with ethidium bromide, and viewed under an ultraviolet trans-illuminator machine. The PCR products with the target band size were purified using MEGAquick-spin™ Total Fragment DNA Purification Kit (Intron Biotechnology, Seoul, Korea). The representa-

Table 1. PCR results and statistical analysis according to origin, gender, breed, age, and health status of dogs

Historical factor	Sample size	No. of positive	Positive rate (%)	x^2	P^*
Origin				15.63	0.0001
Daejeon	50	28	56		
Gyeongbuk	152	39	25.7		
Sex				14.04	0.0002
Male	110	24	21.8		
Female	92	43	46.7		
Breed				17.99	0.0000
Mixed	131	57	43.5		
Pure	71	10	14.1		
Age				15.08	0.0001
≥1 year	168	46	27.4		
<1 year	34	21	61.8		
Health status				23.47	0.0000
Symptomatic	51	31	60.8		
Asymptomatic	151	36	23.8		
Total	202	67	33.2		

*analyzed by Pearson's chi-square test for independence.

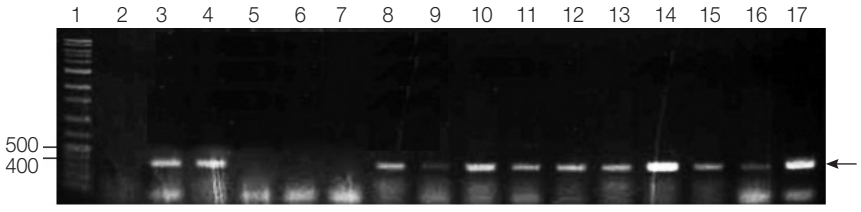

Fig. 1. PCR amplification of *G. intestinalis β-giardin* genes from fecal samples of dogs from animal shelters in Gyeongsangbuk-do and Daejeon. Lane 1: 1,000 bp molecular weight marker, Lanes 2, 5-7: negative samples, Lanes 3-4, 8-16: positive samples, and Lane 17: positive control (*G. intestinalis*, arrow).

tive *β-giardin* gene from 3 fecal samples and a positive control were submitted for sequencing (Fig. 1). The DNA sequences were searched using BLASTN search algorithms (http://www.ncbi.nml.nih.gov/blast/) (Table 2) and aligned using CLUSTAL W2 software (www.ebi.ac.uk/Tools/msa/clustalw2/) (Fig. 2).

Giardins are a family of structural proteins of approximately 29-38 kDa in size and the advantage of using *giardin* genes as targets for molecular detection of *Giardia* cysts is that they are considered unique to this parasite [14]. The prevalence according to the place of origin, gender, breed, age groups, and health status was compared using the chi-square test of Statistical Package for the Social Sciences (SPSS, IBM, Redmond, Washington, USA).

In the present study, the fecal samples collected from stray dogs in animal shelters of Gyeongsangbuk-do and Daejeon showed 25.7% (39 out of 152) and 56% (28 out of 50), respectively, showing that the positive rate was significantly higher in Daejeon ($P<0.0001$) as compared to Gyeongsang-

Table 2. Analysis and comparison of representative *β-giardin* gene from 3 canine fecal samples

Sample	Homology to *G. intestinalis* (%)	Homology to positive control (%)	Description (GenBank accession no.)
Positive control	98	-	*G. intestinalis* assemblage AI *β-giardin* gene (KF963547)
Sample 1	98	91	*G. intestinalis* isolate A21 *β-giardin* gene (AY545647)
Sample 2	98	92	*G. intestinalis* isolate A21 *β-giardin* gene (AY545647)
Sample 3	99	92	*G. intestinalis* isolate BRAdogD15/BRAdogC2 *β-giardin* gene (JF422719)

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sample1         GGGGGAAGCCCTCACGAAG-CTGAAAGAACCTTGAGACCGGCATCGCTACGGAGAACGCC 59
sample2         --GG---GCCCTCAGCAGC-CTGAACG-ACCTCGAGAACGGCATCGCCACGGAGAACGCC 53
sample3         --GG---GCCCTCAAGAACCCTGAACG-ACCTCGAGACCGGCTTCGCCACGGAGAACGCC 54
original        GGGGGAGGGCCCCACGGGC-CTGAAAGAATCTCGAGACGGGCACTGCCACGGAGAACGCA 59
                  **   * ** **  ..  *****.* * ** ****. ***:  ** **********.

sample1         GAGAGGAAGAAGATGTACGACCAGCTCAACGAGAAGGTCGCAGAGGGATTCGCCCGTATT 119
sample2         GAGAGGAAGAAGAAGTACGACCAGCTCAACGAGAAGGTCGCAGAGGGATTCGCCCGTATT 113
sample3         GAGAGGAAGAAGAG-TACGACCAGCTCAACGAGAAGGTCGCAGAGGGATTCGCCCGCATC 113
original        GAAAGGAAGAAGATGTACGACCAGCTCAACGAGAAGGTCGCAGAGGGCTTCGCCCGCATC 119
                **.**********  ********************************.******** **

sample1         TCCGCTGCCATCGAGAAGGAGACGATCGCCCGCGAGAGAGCCGTCAGCGCAGCCACAACA 179
sample2         TCCGCTGCCATCGAGAAGGAGACGATCGCCCGCGAGAGAGCCGTCAGCGCAGCCACAACA 173
sample3         TCCGCCGCCATCGAGAAGGAGACGATCGCCCGCGAGAGGGCCGTCAGCGCAGCCACGACC 173
original        TCCGCCGCGATCGAGAAGGAGACGATCGCCCGCGAGAGGGCCGTTAGCGCTGCCACGACA 179
                ***** ** ****************************.***** *****:*****.**.

sample1         GAGGCTCTCACAAACACGAAGCTCGTCGAGAAGTGCGTCAACGAGCAGCTCGAGAACGTT 239
sample2         GAGGCTCTCACAAACACGAAGCTCGTCGAGAAGTGCGTCAACGAGCAGCTCGAGAACGTT 233
sample3         GAGGCGCTCACAAACACGAAGCTTGTCGAGAAATGCGTCAACGAGCAACTCGAGAACGTC 233
original        GAAGCGCTCACAAACACGAAGCTCGTCGAGAAGTGCGTCAACGAGCAGCTCGAGAACGTC 239
                **.** ***************** ********.**************.***********

sample1         GCCTCGGAGATCCGTGCCATCCAAGAGGAGATCGACCGCGAGAAGGCAGAGCGCAAGGAG 299
sample2         GCCTCGGAGATCCGTGCCATCCAAGAGGAGATCGACCGCGAGAAGGCAGAGCGCAAGGAG 293
sample3         GCCTCGGAGATCCGCGCCATCCAGGAGGAGATCGATCGCGAGAAGGCCGAGCGCAAGGAG 293
original        GCCTCGGAGATCCGCGCTATCCAGGAGGAGATCGACCGCGAGAAGGCCGAACGCAAGGAG 299
                ************** ** *****.*********** ***********.**.*********

sample1         GCAGAGGACAAGATCGTCAACACCCTCGAGGACGTCGTCTCGAAGATCCGGGGCG-GCCT 358
sample2         GCAGAGGACAAGATCGTCAACACCCTCGAGGACGTCGTCTCGAAGATCCGGGGCG-GCCT 352
sample3         GCGGAGGACAAGATCGTTAACACGCTCGAGGACGTCGTCTCGAAGATCCGGGGCGAGCCT 353
original        GCAGAGGACAAGATCGTCAACACTCTCGAGGACGTCGTCTCGAAGATCCGGGGCG-GCCT 358
                **.************** ***** ****************************** ****

sample1         CAA--- 361
sample2         CAAN-- 356
sample3         CAAANN 359
original        CATN-- 362
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Fig. 2. Multiple alignments of representative *β-giardin* genes from fecal samples of dogs from animal shelters in Gyeongsangbuk-do and Daejeon (samples 1, 2, and 3) using CLUSTAL W2 software in comparison to the positive control (original). Asterisks (*) indicate homology among all samples.

buk-do. Sixty-seven out of 202 canine fecal specimens sampled yielded the PCR products of the expected 384 bp fragment of *β-giardin* gene of *G. intestinalis*, showing an overall *Giardia* infection rate of 33.2% (67/202) (Fig. 1). Three of these positive samples and a positive control were sequenced, and all of the nucleotide sequences obtained belonged to *β-giardin* sequences of *G. intestinalis* based on BLASTN search algorithms of the GenBank database (Table 2). The multiple sequence alignment using Clustal W2 software and sequence analysis using BLASTN search algorithms revealed that *β-giardin* gene from the 3 representative positive samples shared 91-92% identity with that of the positive sample (Fig. 2; Table 2). The positive control showed 98% homology to *G. intestinalis* Assemblage A subtype AI, 2 of the positive samples showed 98% homology to *G. intestinalis* Assemblage A, and 1 positive sample showed 99% homology to *G. intestinalis* isolate BRAdogD15/C2 which was reported by Paz e Silva et al. [15] to have a 100% homology to *G. intestinalis* Assemblage C (Table 2).

G. intestinalis Assemblages A and B have a wide range of hosts which include humans and animals such as livestock, dogs, cats, and other wild animals, while Assemblages C and D are dog-specific genotypes [16]. Since *G. intestinalis* Assemblage A has been found in canine fecal samples collected in this study which were similar to those that have been reported [17], the results indicated potential transmission of *Giardia* from dogs to humans or vice versa.

In a study done by Huber et al. [18], neither gender nor age in clinically healthy dogs has a correlation with *Giardia* positive rate although a slightly higher prevalence in male dogs but not significant was reported by Liu et al. [19]. In contrast, the present study showed a significantly higher prevalence in female dogs (46.7%) than in male dogs (21.8%), which is in agreement with those reported by Pallant et al. [20]. Most of the samples tested were obtained from dogs of ≥ 1 year of age (83.2%) but only 27.4% were infected with *Giardia* as compared to the population of younger animals which showed a significant positive rate of 61.8%. This result was similar to those reported by Jacobs et al. [21] where fecal samples from dogs under 1 year of age comprised approximately 73% of *Giardia* cases. Mochizuki et al. [22] reported that the rate of *Giardia*-infected dogs was almost equal in fecal samples obtained from symptomatic or asymptomatic dogs. However, the results in our study showed that the prevalence of dogs with diarrhea was significantly higher than those without diarrhea which was similar to those reported by Liu et al. [19].

In conclusion, *G. intestinalis* Assemblages A and C were found in the feces of dogs from animal shelters in Gyeongsangbuk-do and Daejeon. The prevalence of *G. intestinalis* infection was observed to be higher in females, mixed-breed, < 1 year of age, and symptomatic dogs. Therefore, the role of dogs as a potential source of human giardiasis, though can be considered as minor, could not be excluded, and increasing awareness of this possible transmission to patients or clients is important. Further investigation is recommended to confirm the zoonotic potential of *G. intestinalis* detected in fecal samples from dogs in Korea.

CONFLICT OF INTEREST

The authors of this paper declare no conflict of interest.

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