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Research Brief

Molecular characterization of *Giardia duodenalis* isolates from police and farm dogs in China

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HIGHLIGHTS

- The police and farm dogs have been infected with *Giardia duodenalis* in Shenyang.
- Significantly higher infection rates of farm dogs than police dogs were seen.
- The high occurrence of potentially zoonotic subtype AI-1 in dogs is of public health concern.

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ABSTRACT

To assess the potential zoonotic transmission of giardiasis from dogs in China, a total of 205 fecal specimens from dogs were screened for *Giardia duodenalis* using PCR and sequence analysis of the triosephosphate isomerase gene. The prevalence of *G. duodenalis* in dogs was 13.2% (27/205). The potentially zoonotic assemblage A and the dog-specific assemblage C was identified in 25 (12.2%) and two (1.0%) dogs, respectively. All assemblage A isolates belonged to sub-assemblage AI, genotype AI-1. Likewise, one subtype was found in assemblage C. The high occurrence of potentially zoonotic *G. duodenalis* subtype AI-1 in dogs that are in close contact with humans is of public health concern.

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1. Introduction

Giardiasis is a major diarrheal disease in humans and domestic and wild animals worldwide (Ballweber et al., 2010; Feng and Xiao, 2011; Thompson and Smith, 2011). *Giardia duodenalis* (also known as *Giardia lamblia* or *Giardia intestinalis*) is the species infecting humans and most mammals and consists of at least eight genetically different assemblages A to H. Among them, assemblages A and B infect both humans and many species of animals, thus are considered to be potentially zoonotic, whereas the remaining ones represent host-specific lineages, with assemblages C and D being mostly found in dogs, assemblage E in domestic ruminants and pigs, assemblage F in cats, assemblage G in mice and rats, and assemblage H in marine mammals (Ballweber et al., 2010; Feng and Xiao, 2011; Monis et al., 2003; Thompson and Smith, 2011; Xiao and Fayer, 2008). The contamination of source water by *G. duodenalis* from animal reservoir hosts is of increasing public health concern (Baldursson and Karanis, 2011; Feng et al., 2011; Karanis et al., 2007; Lobo et al., 2009).

Triosephosphate isomerase (*tpi*) gene is a commonly used genetic marker for differentiating *G. duodenalis* isolates at the assemblage and subtype levels (Sprong et al., 2009; Sulaiman et al., 2003; Xiao and Fayer, 2008). For example, in a recent study in the United States, three main assemblages A, C, and D were seen in dogs based on *tpi* sequence analysis (Scorza et al., 2012). Characterization of *G. duodenalis tpi* and other genetic loci has revealed a high incidence of dog-specific assemblages C and D in kennel and household canines in Croatia (Beck et al., 2012). Another study compared three genetic loci (SSU-rDNA, elongation factor 1-alpha, and *tpi*) for genotyping and subtyping *G. duodenalis* isolates and concluded that *tpi* was the most appropriate marker for assessing the zoonotic transmission of *G. duodenalis* between dogs and humans (Traub et al., 2004).

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Thus far, few studies have been conducted to assess the occurrence of *G. duodenalis* genotypes and subtypes in animals in China. Dogs, as the most common companion animals, are in close contact with humans in China. In this study, we have examined the occurrence of *G. duodenalis* in 205 police and farm dogs and characterized the parasite at both the genotype and subtype levels using PCR and sequence analysis of the *tpi* gene.

2. Material and methods

2.1. Ethical considerations

Prior to the collection of canine fecal specimens, permission was obtained from the farm owners and the police canine managers. Dogs were caged, fed alone in each cage and on the following day, fecal specimens were collected in plastic bags. The animals were not harmed in any way during the procedure.

2.2. Specimens

A total of 205 fecal specimens were obtained during October to December 2011 from police and farm dogs in Shenyang, China. Among them, 52 specimens were collected in October and November 2011 from five farms (canine breeding facilities) in suburb and rural areas of Shenyang, where the dogs were free ranging. A second collection of 153 specimens was done in December 2011 at a police canine training station in urban Shenyang, where dogs were kept in cages. Only one specimen per dog was used in the study. All the dogs sampled had frequent contact with their keepers or trainers, and the farm dogs had access to source water. The dogs were assigned into two age groups: adult group with animals older than one year and juvenile group with animals aged between two months to one year.

2.3. Detection of Giardia infection by PCR

Fecal specimens were washed twice in distilled water, and genomic DNA was extracted from 0.3 g of washed specimens using a QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) and manufacturer-recommended procedures. *G. duodenalis* in specimens was detected by nested PCR amplification of a 532-bp fragment of the *tpi* gene as described (Sulaiman et al., 2003). Each specimen was analyzed twice using 2 μl of the DNA extract per PCR. Non-acetylated bovine serum albumin (TaKaRa, Japan) at a concentration of 400 ng/μl was used in primary PCR to neutralize residual PCR inhibitors in the extracted DNA. PCR products were visualized by electrophoresis in 1.5% agarose containing ethidium bromide.

2.4. Genotyping and subtyping of G. duodenalis

The secondary PCR products of the anticipated size were sent to the Sangon Company (Shanghai, China) for DNA sequencing at both directions. The nucleotide sequences obtained were edited using Chromas Pro 1.33 (Technelysium Pty Ltd, Helensvale, Queensland, Australia) and aligned with reference sequences using the ClustalX 1.81 package (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/) to identify *Giardia* species, genotypes, and subtypes.

2.5. Statistical analysis

Statistical analysis was conducted using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Infection rates with *G. duodenalis* were compared using Chi-square analysis at a significance of $p < 0.05$.

3. Results

3.1. G. duodenalis occurrence

Overall, 27 of 205 specimens [13.2%; 95% confidence interval (CI): 0.082–0.181] produced the expected *tpi* PCR product, including 15 (9.8%; 95% CI: 0.048–0.148) from 153 police canines and 12 (23.1%; 95% CI: 0.100–0.361) from 52 farm dogs. The difference in infection rates between farm dogs (23.1%) and police canines (9.8%) was significant ($p < 0.05$). A significantly higher infection rate was seen in adult dogs (26 of 103 or 25.2%; 95% CI: 0.155–0.349) than in juvenile dogs (1 of 102 or 1.0%; 95% CI: −0.009–0.029) ($p < 0.01$). In the police canine training station, 15 of 70 (21.4%; 95% CI: 0.106–0.323) adult dogs examined were positive, whereas none of the 83 juvenile dogs were positive. On farms, 11 of 33 (33.3%; 95% CI: 0.136–0.530) adult dogs and one of 19 (5.3%; 95% CI: −0.051–0.156) juvenile dogs examined were positive ($p < 0.05$). The overall infection rate in female dogs (18/109 or 16.5%; 95% CI: 0.089–0.241) was slightly higher than males (9/96 or 9.4%; 95% CI: 0.033–0.155) ($p > 0.05$). On farms, male dogs (9/35 or 25.7%; 95% CI: 0.089–0.425) had an infection rate higher than females (3/17 or 17.6%; 95% CI: −0.023–0.376) ($p > 0.05$). In the police canine station, 15 of 92 (16.3%; 95% CI: 0.081–0.246) female dogs were positive, whereas none of the 61 male dogs were positive.

3.2. G. duodenalis genotypes and subtypes

Sequence analysis of *tpi* PCR products identified *G. duodenalis* assemblages A and C. The former was detected in 25 (12.2%; 95% CI: 0.074–0.170) specimens, of which 10 were from farm dogs and 15 from police canines. Within assemblage A, one subtype was identified: sub-assemblage AI, genotype AI-1, previously found in humans, cattle, water buffaloes, cats, pigs, dogs, and sheep (Feng and Xiao, 2011). Subtype AI-1 was found in seven male adult dogs, one male juvenile, and two female adult dog on farms and 15 female adult police dogs (Table 1). In contrast, assemblage C was only seen in two (1.0%; 95% CI: −0.004–0.023) specimens from farm dogs (Table 1). The two assemblage C specimens had *tpi* sequences that were identical to a sequence previously identified in a dog in Atlanta, USA (GenBank accession No. AY228643) (Sulaiman et al., 2003) (Table 2). The subtype identified was named as CI-1 in this study (Tables 1 and 2).

4. Discussion

Infection rates of *G. duodenalis* in dogs were reportedly high in some countries: 36.8% in Brazil, 26.6% in Italy, 23.4% in Japan, 22.7% in Belgium, and 21.0% in United Kingdom (Claerebout et al., 2009; Itoh et al., 2011; Paoletti et al., 2008; Upjohn et al., 2010; Volotao et al., 2007). Lower infection rates were reported in some other countries such as the Netherlands (15.2%), Italy (15.0%), Peru (14.5%), China (11.0%), Australia (9.4%), Nicaragua (8.0%), Thailand (7.9%), Finland (5.3%), and Poland (2.0%) (Berrilli et al., 2004; Cooper et al., 2010; Inpankaew et al., 2007; Lebbad et al., 2008; Li et al., 2012; Overgaauw et al., 2009; Palmer et al., 2008; Rimhanen-Finne et al., 2007; Solarczyk and Majewska, 2010). The data obtained in this study clearly showed higher infection rates of *G. duodenalis* in free range dogs on farms than caged dogs in a police canine training station. This may result from the more frequent contact among farm dogs. Most earlier studies have shown lower infection rates in adult animals than in juveniles (Ballweber et al., 2010; Feng and Xiao, 2011; Xiao and Fayer, 2008). Thus, one recent study from China reported that the infection rate of *G. duodenalis* was significantly higher in young pet dogs