Genetic characterization of Cryptosporidium spp. and Giardia duodenalis in dogs and cats in Guangdong, China

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Abstract

Background: There are only limited number of reports on molecular epidemiology of Cryptosporidium spp. and Giardia duodenalis in dogs and cats in China. This study was conducted to assess the infection rates, genetic identity, and public health potential of these parasites in dogs and cats in Guangdong, China.

Methods: PCR and sequence analyses were used to identify and genotype Cryptosporidium spp. and G. duodenalis in fecal samples from 641 dogs and 418 cats in Guangdong. Chi-square test and odds ratio analysis were used to compare the occurrence rates of these pathogens and identify risk factors for infection.

Results: The overall infection rates of Cryptosporidium spp. and G. duodenalis were 6.9% (44/641) and 9.4% (60/641) in dogs, and 6.2% (26/418) and 3.6% (15/418) in cats. Purebred cats (12.4%; $\chi^2 = 5.110, OR = 2.8, P = 0.024$) and dogs (10.8%; $\chi^2 = 5.597, OR = 4.8, P = 0.018$) were more likely to be infected by Cryptosporidium spp. and G. duodenalis, respectively. Dogs (12.0%; $\chi^2 = 7.589, OR = 2.6, P = 0.006$) and cats (13.6%; $\chi^2 = 8.235, OR = 3.5, P = 0.004$) under 6 months had significantly higher infection rates of Cryptosporidium spp. than older animals. Household (13.9%; $\chi^2 = 10.279, OR = 2.6, P = 0.008$) and pet shop dogs (11.0%; $\chi^2 = 7.182, OR = 2.0, P = 0.048$) had higher occurrence of Cryptosporidium spp., as was the case for G. duodenalis occurrence in experimental dogs (13.4%; $\chi^2 = 9.223, OR = 1.9, P = 0.017$). Cryptosporidium canis ($n = 42$), C. muris ($n = 1$) and Cryptosporidium rat genotype IV ($n = 1$) were identified in dogs, while C. felis ($n = 21$), C. parvum ($n = 3$), C. muris ($n = 1$) and Cryptosporidium rat genotype IV ($n = 1$) were identified in cats. In contrast, the canine-specific assemblages C ($n = 27$) and D ($n = 26$) and the feline-specific assemblage F ($n = 14$) were almost exclusively the only genotypes of G. duodenalis in dogs and cats, respectively. There was no significant difference in infection rates of Cryptosporidium spp. and G. duodenalis between diarrheal and non-diarrheal pets.

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**Background**

Cryptosporidium spp. and *Giardia duodenalis* are important protozoan parasites that inhabit the gastrointestinal tract of humans and other vertebrates. Diarrhea is the main clinical symptom of cryptosporidiosis and giardiasis. Humans acquire these two pathogens through contact with infected persons and animals, or consuming contaminated food or water [1, 2]. Among the ~40 known Cryptosporidium species, *C. hominis*, *C. parvum*, *C. meleagrisidis*, *C. canis* and *C. felis* are the most common species in humans [3]. Similarly, among the eight common genotypes (A to H) of *G. duodenalis*, only assemblages A and B are major human pathogens [2].

Cryptosporidium spp. and *G. duodenalis* are commonly detected in dogs and cats worldwide [4, 5]. *Cryptosporidium canis* and *C. felis* are major Cryptosporidium species in dogs and cats respectively, but *C. hominis*, *C. parvum*, *C. muris* and *C. ubiquitum* have been occasionally detected in these animals [6–10]. Similarly, dog-adapted assemblages C and D, and cat-adapted assemblage F are the dominant *G. duodenalis* genotypes in these animals, although zoonotic assemblages A and B have been identified in some studies [2, 11].

Limited data are available on the transmission of Cryptosporidium spp. and *G. duodenalis* in dogs and cats in China. The reported infection rates of Cryptosporidium spp. range from 1.6% to 10.5%, with *C. canis* and *C. felis* being identified as the dominant Cryptosporidium species in dogs and cats, respectively. In contrast, the infection rates of *G. duodenalis* were reported to range from 1.9 to 26.2%, with assemblages A, B, C, D and E being identified in dogs and assemblage F in cats [9, 11–21]. The risk factors involved in the acquisition of cryptosporidiosis and giardiasis have rarely been examined in these studies.

Guangdong Province has the largest populations of humans (111.69 million in 2017) [22] and pets (10.62% of the > 100 million pets in the country in 2015 were in Guangdong [23] in China. The subtropical climate and abundant rainfall provide a favorable environment for the transmission of waterborne pathogens such as Cryptosporidium spp. and *G. duodenalis*. Both cryptosporidiosis and giardiasis are known to be common in AIDS patients and diarrheic children in Guangdong, China [24, 25]. Several studies have also reported the prevalence of *G. duodenalis* in dogs and cats in the province [12, 15, 18].

Thus far, there are no systematic studies of Cryptosporidium spp. in dogs and cats in the province. As children in China are sometimes infected with several zoonotic Cryptosporidium species (*C. canis* and *C. felis*) that are traditionally associated with pets [26, 27], we examined in this study the occurrence and identity of Cryptosporidium spp. and *G. duodenalis* in dogs and cats in Guangdong for the assessment of the zoonotic potential of these pathogens.

**Conclusions:** While domestic pets in Guangdong are infected with zoonotic Cryptosporidium species, they are mainly infected with host-specific *G. duodenalis* genotypes. Risk factors for infections differ between Cryptosporidium spp. and *G. duodenalis* and between dogs and cats.

**Keywords:** Cryptosporidium spp., Giardia duodenalis, Genotype, Risk factors
**Methods**

**Sample collection**
From July 2017 to August 2018, 1059 fecal samples were collected from dogs and cats in five cities of Guangdong (Fig. 1). Among them, 641 were from dogs of various living settings, including households (n = 79), veterinary clinics (n = 109), pet shelters (n = 134), pet shops (n = 118) and a research center (n = 201). Simultaneously, 418 fecal samples were collected from cats in households (n = 49), veterinary clinics (n = 130), pet shelters (n = 132), pet shops (n = 27), and strays (n = 80) in these cities. The animals were divided into two age groups: ≤ 6 months (125 dogs and 66 cats); and > 6 months (402 dogs and 299 cats), with 114 dogs and 53 cats of unknown age. In addition, we recorded information on the sex (291 and 129 female dogs and cats, 191 and 163 male dogs and cats, respectively, and 159 dogs and 126 cats of unknown sex), breed (446 and 89 purebred dogs and cats, 82 and 187 mixed-breed dogs and cats, respectively, and 113 dogs and 142 cats of unknown breeds) and clinical signs (17 and 19 diarrheic dogs and cats, and 624 and 399 non-diarrheic dogs and cats, respectively) of the animals as conditions permitted. Each fecal sample was placed into a 50 ml plastic centrifuge tube with 2.5% potassium dichromate, transferred to the laboratory, and stored at 4 °C for less than two weeks before DNA extraction.

**DNA extraction and PCR analysis**
Each fecal sample was washed twice with distilled water by centrifugation. DNA was extracted from the washed fecal materials using a Fast DNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). The extracted genomic DNA was stored at -20 °C until use. A nested PCR targeting the small subunit (SSU) rRNA gene was employed to detect Cryptosporidium spp. [28], while PCR assays targeting the β-giardin (bg) [29], glutamate dehydrogenase (gdh) [30] and triosephosphate isomerase (tpi) [31] genes were employed to detect G. duodenalis. An ~850-bp fragment of 60 kDa glycoprotein (gp60) gene was amplified to identify the subtype of C. parvum [32]. Each sample was analyzed at least twice by PCR at each genetic locus, with both negative and positive controls being included in each PCR analysis. The secondary PCR products were analyzed by 1.5% agarose electrophoresis.

**Sequence analysis**
All secondary PCR products of the expected size were sequenced on an ABI3730 autosequencer by the Sangon Biotech (Shanghai, China) in both directions using the PCR primers. The DNA sequences obtained were assembled using ChromasPro 1.5 (http://www.Technelysi um.com.au/ChromasPro.html) and edited using BioEdit 7.1.3.0 (http://www.mbio.ncsu.edu/BioEd it/bioed it.html). They were aligned with reference sequences of each locus downloaded from GenBank using Clustal X 2.1 (http://www.clustal.org/) to determine the identity of Cryptosporidium species and G. duodenalis genotypes.

**Statistical analysis**
Differences in infection rates of Cryptosporidium spp. and G. duodenalis in dogs and cats were compared between sexes, breeds, age groups, living conditions and clinical signs using the Chi-square test implemented in SPSS 20.0 version (IBM Inc., Chicago, IL, USA). Odds
ratios (OR) and their 95% confidence intervals (95% CI) were calculated to identify risk factors involved in the acquisition of these pathogens. Differences were considered significant at \( P < 0.05 \).

**Results**

**Occurrence and risk factors of Cryptosporidium infection in dogs and cats**

*Cryptosporidium* spp. were detected by PCR in 44 (6.9%) of the 641 canine samples and 26 (6.2%) of the 418 feline samples (Tables 1 and 2). Odds ratios analysis identified some risk factors involved in the transmission of *Cryptosporidium* spp. in dogs and cats. Dogs (12.0%; \( \chi^2 = 7.589; \ OR = 2.6; \ P = 0.006 \)) and cats (13.6%; \( \chi^2 = 8.235; \ OR = 3.5; \ P = 0.004 \)) aged under 6 months were at higher risk of *Cryptosporidium* infection. Purebred cats were more susceptible to *Cryptosporidium* (12.4%; \( \chi^2 = 5.597; \ OR = 2.8; \ P = 0.024 \)) infection. Household (13.9%; \( \chi^2 = 10.279; \ OR = 2.6; \ P = 0.008 \)) and pet shop dogs (11.0%; \( \chi^2 = 7.182; \ OR = 2.0; \ P = 0.048 \)) were more likely to be infected by *Cryptosporidium* spp. In contrast, there were no significant impacts on infection rates of *Cryptosporidium* spp. by sex or breed of dogs, and sex and living condition of cats (Tables 1 and 2).

**Occurrence and risk factors of G. duodenalis infection in dogs and cats**

*Giardia duodenalis* was detected in 60 (9.4%) of the 641 canine samples and 15 (3.6%) of the 418 feline samples (Tables 1 and 2). The infection rate in female dogs (12.4%) was significantly higher than in male dogs (6.3%; \( \chi^2 = 4.767; \ OR = 2.1; \ P = 0.029 \)) (Table 1), while the infection rate in male cats (5.5%) was significantly higher than in female cats (0; \( \chi^2 = 7.349; \ OR = 112.2; \ P = 0.001 \)) (Table 2). Purebred dogs (10.8%) had a higher infection rate of *G. duodenalis* than mixed breed dogs (2.4%; \( \chi^2 = 5.597; \ OR = 4.8; \ P = 0.018 \)). The infection rates in household (10.1%), pet shop (10.2%) and research dogs (13.4%) were significantly higher than in dogs in veterinary clinics (2.8%; \( \chi^2 = 4.522; \ P = 0.033 \); \( \chi^2 = 5.051; \ P = 0.025 \); \( \chi^2 = 9.223; \ OR = 1.9; \ P = 0.017 \) respectively) (Table 1). Cats from veterinary clinics (8.0%) had a significantly higher infection rate than stray cats (0%; \( \chi^2 = 5.118; \ P = 0.024 \)) (Table 2).

**Table 1** Infection rates of *Cryptosporidium* spp. and *Giardia duodenalis* in dogs by sex, breed, age, sample source and clinical signs

| Variable                  | n  | *Cryptosporidium* spp. | G. duodenalis |          |          |          |
|---------------------------|----|------------------------|---------------|---------|---------|---------|
|                           |    | No. positive (%)       | OR (95% CI)   | P-value | No. positive (%) | OR (95% CI)  | P-value |
| Sex                       |    |                        |               |         |          |         |
| Female                    | 291| 22 (7.6)               | 1.7 (0.7–3.7) | 0.213   | 36 (12.4) | 2.1 (1.1–4.2) | 0.029*  |
| Male                      | 191| 9 (4.7)                | 0.6 (0.3–1.3) |         | 12 (6.3)  | 0.5 (0.2–0.9)  |         |
| Unknown                   | 159| 13 (8.2)               |               |         | 12 (7.5)  |               |         |
| Breed                     |    |                        |               |         |          |         |
| Purebred                  | 446| 29 (6.5)               | 1.1 (0.4–2.9) | 0.891   | 48 (10.8) | 4.8 (1.1–20.3) | 0.018*  |
| Mixed-breed               | 82 | 5 (6.1)                | 0.9 (0.3–2.5) |         | 2 (2.4)   | 0.2 (0.0–0.9)  |         |
| Unknown                   | 113| 10 (8.8)               |               |         | 10 (8.8)  |               |         |
| Age (months)              |    |                        |               |         |          |         |
| ≤ 6                       | 125| 15 (12.0)              | 2.6 (1.3–5.3) | 0.006** | 14 (11.2) | 1.3 (0.7–2.5)  | 0.455   |
| > 6                       | 402| 20 (5.0)               | 0.4 (0.2–0.8) |         | 36 (9.0)  | 0.8 (0.4–1.5)  |         |
| Unknown                   | 114| 9 (7.9)                |               |         | 10 (8.8)  |               |         |
| Sample source             |    |                        |               |         |          |         |
| Household                 | 79 | 11 (13.9)              | 2.6 (1.3–5.4) | 0.008** | 8 (10.1)  | 1.1 (0.5–2.4)  | 0.803   |
| Pet shop                  | 118| 13 (11.0)              | 2.0 (1.0–3.9) | 0.048*  | 12 (10.2) | 1.1 (0.6–2.2)  | 0.738   |
| Pet shelter               | 134| 9 (6.7)                | 1.0 (0.5–2.1) | 0.939   | 10 (7.5)  | 0.7 (0.4–1.5)  | 0.397   |
| Research center           | 201| 7 (3.5)                | 0.4 (0.2–0.9) | 0.022*  | 27 (13.4) | 1.9 (1.1–3.3)  | 0.017*  |
| Veterinary clinic          | 109| 4 (3.7)                | 0.5 (0.2–1.3) | 0.148   | 3 (2.8)   | 0.2 (0.1–0.8)  | 0.009** |
| Clinical signs            |    |                        |               |         |          |         |
| Diarrheic                 | 17 | 2 (11.8)               | 1.8 (0.4–8.3) | 0.418   | 2 (11.8)  | 1.3 (0.3–5.8)  | 0.730   |
| Non-diarrheic             | 624| 42 (6.7)               | 0.5 (0.1–2.4) |         | 58 (9.3)  | 0.8 (0.2–3.4)  |         |
| Total                     | 641| 44 (6.9)               |               |         | 60 (9.4)  |               |         |

*Abbreviation: n; total number of samples  
* \( P < 0.05 \), ** \( P < 0.01 \)
Distribution of Cryptosporidium species

The secondary PCR products from all 44 Cryptosporidium-positive canine and 26 Cryptosporidium-positive feline samples were sequenced successfully. Among the canine samples, 42 were identified as positive for C. canis, and one each for C. muris and the Cryptosporidium rat genotype IV. Among the feline samples, 21 were identified as positive for C. felis, three for C. parvum, and one each for C. muris and Cryptosporidium rat genotype IV (Table 3).

Within C. canis, the nucleotide sequences of the SSU rRNA gene obtained from 21 samples were identical to the GenBank reference sequence KJ776591, while nucleotide sequences from the remaining 21 C. canis samples had minor differences from the reference sequence, including one single nucleotide polymorphism (SNP) in 20 samples (T to C substitution at position 627 of KJ776591), four SNPs in one sample (A to G substitution at positions 293 and 341, and T to C substitution at positions 561 and 627 of KJ776591). Within C. felis, the nucleotide sequences obtained from 18 samples were identical to the GenBank reference sequence KM977642, while those from the remaining three samples were identical to the reference sequence AF159113. Within C. parvum, two nucleotide sequences were identical to the reference sequence AB968048, whereas the third one had two SNPs compared to the reference sequence (T to C substitution at position 102, and G to A substitution at position 586 of AB968048). The nucleotide sequence from C. muris in the feline sample was identical to KM870575, while the one from the canine sample had two SNPs (C to G substitution at position 112, and G to A substitution at position 196). Within the Cryptosporidium rat genotype IV, the sequence obtained from the canine sample had two SNPs (T to C substitution at positions 360 and 418) compared to AY737582, while the other one from the feline sample had one SNP (A to G substitution at position 427) (Additional file 1: Table S1).

Distribution of Giardia duodenalis assemblages

Fifty-eight of the 60 G. duodenalis-positive samples from dogs and all 15 G. duodenalis-positive samples from cats were sequenced successfully. There were some differences in the PCR detection rates among the bg, tpi, and gdh loci (Additional file 1: Table S2). Giardia duodenalis assemblages C, D and concurrence

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Table 2 Infection rates of Cryptosporidium spp. and Giardia duodenalis in cats by sex, breed, age, sample source and clinical signs

| Variable                  | n   | Cryptosporidium spp. |          | G. duodenalis |          |
|---------------------------|-----|----------------------|----------|--------------|----------|
|                           |     | No. positive (%)     | OR (95% CI) | P-value | No. positive (%) | OR (95% CI) | P-value |
| Sex                       |     |                      |          |              |          |
| Female                    | 129 | 11 (8.5)             | 1.4 (0.6–3.5) | 0.433 | 0              | 112.2 (0.2–5.7 × 10⁴) | 0.001** |
| Male                      | 163 | 10 (6.1)             | 0.7 (0.3–1.7) | 0.277 | 9 (5.5)        | 0 (1.7 × 10⁻³–4.6)  | 0.968  |
| Unknown                   | 126 | 5 (4.0)              | 6 (4.8)   | 0.993 | 6 (4.8)        | 1 (0.2–4.8)       | 0.993  |
| Breed                     |     |                      |          |              |          |
| Purebred                  | 89  | 11 (12.4)            | 2.8 (1.1–7.0) | 0.024* | 1 (1.1)       | 1.1 (0.1–11.7)    | 0.968  |
| Mixed-breed               | 187 | 9 (4.8)              | 0.4 (0.1–0.9) | 0.004** | 2 (3.0)      | 1.0 (0.2–4.8)     | 0.993  |
| Unknown                   | 142 | 6 (4.2)              | 12 (8.4)  | 0.993 | 12 (8.4)       | 4 (7.5)          | 0.993  |
| Age (months)              |     |                      |          |              |          |
| ≤ 6                       | 66  | 9 (13.6)             | 3.5 (1.4–8.5) | 0.004** | 2 (3.0)      | 1.0 (0.2–4.8)     | 0.993  |
| > 6                       | 299 | 13 (4.3)             | 0.3 (0.1–0.7) | 0.993 | 9 (3.0)       | 1.0 (0.2–4.7)     | 0.993  |
| Unknown                   | 53  | 4 (7.5)              | 4 (7.5)   | 0.993 | 4 (7.5)       | 4 (7.5)          | 0.993  |
| Sample source             |     |                      |          |              |          |
| Household                 | 49  | 1 (2.0)              | 0.3 (0.0–2.2) | 0.198 | 1 (2.0)      | 0.6 (0.1–4.3)     | 0.573  |
| Pet shop                  | 27  | 3 (1.1)              | 2.0 (0.6–7.1) | 0.277 | 0            | 0 (1.2 × 10⁻⁶–50.6) | 0.370  |
| Pet shelter               | 132 | 7 (5.4)              | 0.8 (0.3–1.9) | 0.598 | 6 (4.7)      | 1.3 (0.5–3.4)     | 0.617  |
| Veterinary clinic          | 130 | 10 (7.7)             | 1.4 (0.6–3.2) | 0.403 | 8 (8.0)      | 1.8 (0.7–4.3)     | 0.203  |
| Stray                     | 80  | 5 (6.3)              | 1.0 (0.4–2.8) | 0.990 | 0            | 0 (6.7 × 10⁻⁵–16.9) | 0.098  |
| Clinical signs            |     |                      |          |              |          |
| Diarrheic                 | 19  | 1 (5.3)              | 0.8 (0.1–6.5) | 0.860 | 0            | 0.1 (2.6 × 10⁻⁴–68.8) | 0.46   |
| Non-diarrheic             | 399 | 25 (6.3)             | 1.2 (0.2–9.4) | 15 (3.8) | 7.4 (1.4 × 10⁻²–3.8 × 10³) | 0.004** |
| Total                     | 418 | 26 (6.2)             | 15 (3.6)  | 0.993 | 15 (3.6)      | 7.4 (1.4 × 10⁻²–3.8 × 10³) | 0.004** |

Abbreviation: n; total number of samples
* P<0.05, ** P<0.01
of both were detected in 27, 26 and 5 dogs, respectively. In contrast, assemblages A and F were found in 1 and 14 cats, respectively (Table 3). The assemblage A was identified as A1 (GenBank: L40509) at the gdh locus, A5 (GenBank: AB469365) at the bg locus, and A4 (GenBank: GQ329677) at the tpi locus, for assemblage F, the nucleotide sequences from seven samples were identical to AB469365 at the gdh locus, identified as A1 (GenBank: L40509) at the bg locus, and A4 (GenBank: GQ329677) at the tpi locus. The nucleotide sequence differences within G. duodenalis assemblages C and D at the bg, gdh and tpi loci are shown in Additional file 1: Table S3.

**Table 3** Species/genotypes/assemblages of Cryptosporidium spp. and Giardia duodenalis in dogs and cats by sex, breed, age, sample source and clinical signs

| Variable       | Dogs                        | Cats                        |
|----------------|-----------------------------|-----------------------------|
|                | n  | Cryptosporidium genotype (n) | G. duodenalis assemblage (n) | n  | Cryptosporidium genotype (n) | G. duodenalis assemblage (n) |
| Sex            |    |                             |                             |    |                             |                             |
| Female         | 291 | C. canis (21); rat genotype IV (1) | C (19); D (12); C/D (4) | 129 | C. felis (10); C. parvum (1) |                             |
| Male           | 191 | C. canis (9)                | C (4); D (6); C/D (1)      | 163 | C. felis (7); C. parvum (1); C. muris (1); rat genotype IV (1) |                             |
| Unknown breed  | 159 | C. canis (12); C. muris (1) | C (4); D (8)               | 126 | C. felis (4); C. parvum (1) | F (6)                      |
| Purebred       | 446 | C. canis (28); C. muris (1)  | C (23); D (18); C/D (5)    | 144 | C. felis (4); C. parvum (2) | A (1); F (11)              |
| Mixed-breed    | 82  | C. canis (4); rat genotype IV (1) | C (2)                     | 187 | C. felis (9)                | F (2)                      |
| Unknown age (months) |      |                             |                             |    |                             |                             |
| ≤ 6            | 125 | C. canis (14); C. muris (1)  | C (9); D (4); C/D (1)      | 66  | C. felis (7); C. parvum (1); rat genotype IV (1) | A (1); F (1)              |
| > 6            | 402 | C. canis (19); rat genotype IV (1) | C (16); D (14); C/D (4)    | 299 | C. felis (11); C. parvum (1); C. muris (1) | F (9)                      |
| Unknown sample source |      |                             |                             |    |                             |                             |
| Household      | 79  | C. canis (11)               | C (2); D (8)               | 53  | C. felis (3); C. parvum (1) | F (4)                      |
| Pet shop       | 118 | C. canis (12); C. muris (1)  | C (4); D (3); C/D (1)      | 49  | C. felis (1)                | F (1)                      |
| Pet shelter    | 134 | C. canis (9)                | C (8); D (3); C/D (1)      | 27  | Rat genotype IV (1); C. felis (2) |                             |
| Research center| 201 | C. canis (7)                | C (2); D (8)               | 132 | C. felis (6); C. parvum (1) | F (6)                      |
| Veterinary clinic | 109 | C. canis (3); rat genotype IV (1) | C (13); D (11); C/D (3)    | 0   | Rat genotype IV (1); C. felis (2) |                             |
| Stray          | 0   | –                           | –                          | 130 | C. felis (7); C. parvum (2); C. muris (1) | A (1); F (7)              |
| Clinical signs |      |                             |                             | 80  | C. felis (5)                | –                          |
| Diarrheic      | 17  | C. canis (2)                | C (1); D (1)               | 19  | C. felis (1)                | –                          |
| Non-diarrheic  | 624 | C. canis (40); C. muris (1); rat genotype IV (1) | C (26); D (25); C/D (5) | 399 | C. felis (20); C. parvum (3); C. muris (1); rat genotype IV (1) | A (1); F (14)             |
| Total          | 641 | C. canis (42); C. muris (1); rat genotype IV (1) | C (27); D (26); C/D (5) | 418 | C. felis (21); C. parvum (3); C. muris (1); rat genotype IV (1) | A (1); F (14)             |

**Concurrent infections of Cryptosporidium spp. and G. duodenalis**

Co-infection of Cryptosporidium spp. and G. duodenalis was found in 13 dogs and 1 cat. Among them, 9 dogs had co-infection of C. canis and assemblage C, 4 dogs had co-infections of C. canis and assemblage D, and one cat had co-infection of C. parvum and assemblage A. The co-infection rate in household dogs (6.3%) was significantly higher than in pet shelters (0.75%; \( \chi^2 = 5.659, P = 0.017 \)).

**Discussion**

We have shown in the present study a common occurrence of Cryptosporidium spp. and G. duodenalis in dogs and cats in five cities in Guangdong. Young age
was identified as the main risk factor for the transmission of Cryptosporidium spp. in these animals. The finding of higher infection rates of Cryptosporidium spp. in dogs and cats under 6 months is consistent with previous studies conducted elsewhere [14, 33].

Results of the present study suggest that Cryptosporidium spp. and G. duodenalis have different transmission characteristics between dogs and cats. For example, young age was identified as a risk factor for the transmission of Cryptosporidium spp., but not for G. duodenalis for both dogs and cats; pure breed was a risk factor for Cryptosporidium spp. in cats and G. duodenalis in dogs; and the female and male sexes were risk factors for G. duodenalis in dogs and cats, respectively. Previous studies have shown that pedigree pets were more susceptible to infectious diseases [34, 35]. Consistent with these observations, household and pet shop dogs had higher infection rate of Cryptosporidium spp. than dogs in research centers, while the opposite was observed for G. duodenalis. Some of the differences are attributable to the different life styles between dogs and cats; domestic cats mostly stay indoors and have little chance of contact with other pets or contaminated environment. In contrast, pet owners in urban areas often exercise their dogs in parks, where dogs frequently have contact with other pets and contaminated soil, increasing the risk of transmission of these parasites in household dogs [36, 37]. The higher infection rates of these parasites in pet shop and experimental dogs are expected. These places are often overcrowded with young animals and have inadequate sanitary control, which may provide a favorable environment for the fecal-oral transmission of Cryptosporidium spp. and G. duodenalis [38, 39].

Results of the present study support the suggestion that C. canis and C. felis are the most common Cryptosporidium species in dogs and cats, respectively [10]. As members of the five most common human-pathogenic Cryptosporidium species, C. canis and C. felis have been detected in humans worldwide [40–42], sometimes in both pets and their owners [43, 44]. The other zoonotic species C. parvum detected in three cats in the present study had been reported in pets previously [9, 45]. Subtype analysis had identified the common C. parvum subtypes IIA15G2R1 and IIA17G2R1 in urban companion animals in Great Britain [46, 47]. We have failed to subtype the C. parvum in the present study, thus cannot exclude the possibility of the transient passage of the parasite without established infection. The occasional infections of C. muris in pets are expected, as this Cryptosporidium species is common in rodents in China [48]. Dogs and cats are in frequent contact with rodents either in pet shops or in the wild.

Giardia duodenalis assemblages C, D, and F were the most prevalent genotypes in this study. These results are consistent with the observation in previous studies that these assemblages are the most common genotypes in dogs and cats [49]. In addition, assemblage A infection was detected in one cat from a veterinary clinic, and this genotype was previously found in cats in Guangzhou [18]. Even though assemblages A and B are the main zoonotic genotypes, assemblages C, D, and F have been identified in a few human cases [50–53].

Conclusions
Results of this study suggest a common occurrence of Cryptosporidium spp. and G. duodenalis in dogs and cats in Guangdong, China, and young age, certain sex, pure breed and some living conditions could be risk factors for infections. Most Cryptosporidium species detected in the study, namely C. canis, C. félis, C. parvum and C. muris, are known zoonotic parasites while almost all of the G. duodenalis genotypes in dogs and cats are host-adapted ones. Further studies with sampling of humans and pets in the same area and characterization of zoonotic Cryptosporidium spp. at the subtype level are needed for improved understanding of zoonotic transmission of Cryptosporidium spp. in humans due to contact with pets.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s13071-019-3822-z.

Additional file 1: Table S1. Nucleotide substitutions in partial sequences of the SSU rRNA gene of Cryptosporidium species/genotypes obtained from dogs and cats in Guangdong. Table S2. Occurrence rates of G. duodenalis by PCR analyses of the β-giardin, glutamate dehydrogenase, and triosephosphate isomerase genes in dogs and cats. Table S3. Nucleotide substitutions in partial sequences of the β-giardin, glutamate dehydrogenase, and triosephosphate isomerase genes of G. duodenalis assemblages obtained from dogs in Guangdong.

Abbreviations
PCR: polymerase chain reaction; SSU rRNA: small subunit rRNA; bg: β-giardin; gdh: glutamate dehydrogenase; tpi: triosephosphate isomerase; SNP: single nucleotide polymorphism.

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Authors’ contributions
YF and LX conceived and designed the experiments. JL, XD and KZ performed the experiments. JL, NL, YG and ZZ analyzed the data. JL, YF and LX wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials
Data supporting the conclusions of this article are included within the article. Representative DNA sequences from the present study were deposited in the GenBank database under accession numbers MN272322-MN272327 for Cryptosporidium spp., and MN270280-MN270301 for G. duodenalis.

Ethics approval and consent to participate
The fecal samples used in this study were collected with the permission of the owners of the pets. Freshly excrated fecal materials were collected with the assistance of the pet owners without direct handling of the animals. The research protocol was reviewed and approved by the Research Ethics Committee of the South China Agricultural University.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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