VETERINARSKI ARHIV 95 (2), 197-210, 2025 DOI: 10.24099/vet.arhiv.2406

Molecular detection of *Cryptosporidium* and *Giardia duodenalis* in canine faecal samples in the Qinghai Tibetan Plateau area, China

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JIAN, Y., X. ZHANG, L. MA, G. WANG, Q. CAI, G. WANG, X. LI, P. KARANIS: Molecular detection of *Cryptosporidium* and *Giardia duodenalis* in canine faecal samples in the Qinghai Tibetan Plateau area, China. Vet. arhiv 95, 197-210, 2025.

ABSTRACT

Dogs act as natural reservoirs of a large number of zoonotic pathogens, including the intestinal *Cryptosporidium* spp. and *Giardia duodenalis*, the most relevant protozoan species causing gastrointestinal diseases worldwide. An epidemiological study aiming to assess the prevalence and molecular diversity of *Cryptosporidium* and *G. duodenalis* was conducted in the Qinghai Tibetan Plateau area (QTPA), Northwest China. A total of 217 dog faecal samples were collected from sheep farms, dog farms and pet hospitals. The species/genotypes of *Cryptosporidium* and *G. duodenalis* isolated from dogs were identified by the PCR-based method targeting the partial 18S ribosomal RNA gene for *Cryptosporidium* and β-giardin gene for *G. duodenalis*. The results of *Cryptosporidium* spp. and *G. duodenalis* infections in dogs showed an overall prevalence of 2.8% (6/217) and 6.9% (15/217), respectively. No *Cryptosporidium* and *G. duodenalis* co-infections were observed. The PCR-sequence results confirmed detection of *C. parvum* (n=3), *C. canis* (n=2), *C. andersoni* (n=1) and *G. duodenalis* assemblages B (n=9), C (n=3) and D (n=3) in dogs from the QTPA. The results of the present study demonstrated that dogs in the QTPA are commonly exposed to *Cryptosporidium* spp. and *G. duodenalis*. This may indicate that dogs are potential sources of infection of *Cryptosporidium* spp. and *G. duodenalis* transmitting to humans and animals.

Key words: *Cryptosporidium*; *Giardia duodenalis*; dogs; prevalence; molecular detection; Qinghai Tibetan Plateau Area

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ISSN 0372-5480
Printed in Croatia

Introduction

Cryptosporidium spp. and *G. duodenalis* are common zoonotic protozoan parasites that can infect a wide variety of vertebrate hosts, including humans, and domestic and wild animals worldwide, causing acute or chronic diarrhoeal illness (KHAN and WITOLA, 2023; TAWANA et al., 2023; ZUO et al., 2023). Cryptosporidiosis and giardiasis are waterborne and foodborne diseases transmitted via direct or indirect faecal-oral routes, where the hosts become infected by ingesting contaminated water and/or food (BALDURSSON and KARANIS, 2011; AHMED and KARANIS, 2018). At least 44 *Cryptosporidium* spp. have been reported, and more than 120 genotypes have been recognized (RYAN et al., 2014; RYAN and HIJJAWI, 2015; KOEHLER et al., 2016; KVAC et al., 2016; RYAN et al., 2016; ZAHEDI et al., 2016). Eight *G. duodenalis* assemblages (A-H), have been identified (FENG and XIAO, 2011; THOMPSON and ASH, 2019). In rural areas, especially in the pastures and living environments of the herders in the Qinghai Tibetan Plateau Area (QTPA), dogs share a close relationship with humans. They are greatly valued for companionship, recreation and protection. Several studies in China (JIAN et al., 2014; LI et al., 2015; QI et al., 2016a; XU et al., 2016; ZHANG et al., 2016b; YU et al., 2018) and worldwide (GIL et al., 2017; TANGTRONGSUP et al., 2017; ADELL-ALEDON et al., 2018; ALVES et al., 2018; GHARIEB et al., 2018; MUGALA et al., 2018; ROSANOWSKI et al., 2018; GODINEZ-GALAZ et al., 2019) have demonstrated that some dogs are infected with *Cryptosporidium* spp. and *G. duodenalis*, and that they can be important reservoirs of these two species of parasites, with a significant impact on public health for humans and animals. These two protozoas have an especially serious effect on immunocompromised individuals and children.

Among the 44 *Cryptosporidium* species recognized, *Cryptosporidium canis* is the host-adapted species responsible for most cryptosporidiosis cases in dogs, and it is also a zoonotic species infecting human. Prevalence studies have confirmed that *C. parvum*, *C. andersoni*, *C. hominis*, *C. ubiquitum* and *C. muris* are also found in dogs from various regions worldwide (LI et al., 2015; GIL et al., 2017; ALVES et al., 2018; GHARIEB et al., 2018; ROSANOWSKI et al., 2018). Regarding *G. duodenalis*, eight (A to H) genetically distinct assemblages have been defined, whereby the host-adapted assemblages C and D are usually detected in dogs, the zoonotic assemblages A and B are found in both humans and animals, assemblage E in ruminants, and assemblage F in cats (BALLWEBER et al., 2010; FENG and XIAO, 2011; THOMPSON and ASH, 2019). Likewise, the sub-assemblages AI, AII, AIII, BIII, and BIV, and assemblages E and F have also been reported in dogs (LI et al., 2015; GIL et al., 2017; ADELL-ALEDON et al., 2018; PAN et al., 2018; YU et al., 2018; GODINEZ-GALAZ et al., 2019).

Several prevalence studies have been published on *Cryptosporidium* spp. infections of various dog populations in different regions of China, such as the *C. canis* dog genotype in raccoon dogs in Heilongjiang, Jilin, and Liaoning Provinces (YANG et al., 2018), *C. canis* in companion dogs in Beijing (YU et al., 2018), *C. canis* in raccoon dogs in suburban Harbin (ZHANG et al., 2016a), *C. canis* in pet, clinic and household dogs in Shanghai (XU et al., 2016), *C. canis* and *C. ubiquitum* in dogs in Heilongjiang (LI et al., 2015) and *C. canis* in dogs in Henan (JIAN et al., 2014). Similarly, *G. duodenalis* has also been reported in different regions of China, for example, *G. duodenalis* assemblages AI, C and D in stray dogs in Guangdong (PAN et al., 2018), *G. duodenalis* assemblages C, D and F in companion dogs in Beijing (YU et al., 2018), C and D in stray dogs in Sichuan (ZHANG et al., 2017), C and D in pet dogs in Shanghai (LIU et al., 2017), C and D in raccoon dogs in Jilin, Heilongjiang, Liaoning, Hebei and Shandong (ZHANG et al., 2016b), A, B, C and D in dogs in Shanghai (XU et al., 2016), C and D in pet and stray dogs in Henan (QI et al., 2016a), C and E in dogs in Heilongjiang (LI et al., 2015), A, C and D in dogs in Guangdong (ZHENG et al., 2014), AI and C in police and farm dogs in Shenyang (LI et al., 2013) and A and D in pet dogs in Guangzhou (LI et al., 2012).

Notably, these prevalence and molecular studies have identified and demonstrated the separate occurrence of the two parasitic pathogens in dog populations, suggesting that infected dogs may be suitable reservoirs for further infections. However, there are no data showing co-infection of *Cryptosporidium* and *G. duodenalis* in dogs in the QTPA of China. The present study was undertaken to determine the prevalence of *Cryptosporidium* and *G. duodenalis* species/genotypes/assemblages in dogs from this region, and to estimate the potential role of dogs in zoonotic transmission of cryptosporidiosis and giardiasis.

Materials and methods

The study sites. The faecal samples were collected from dogs in different locations in the QTPA of China (Fig. 1 and Table 1). The sampling sites were located on sheep farms composed of family pastures, dog farms and pet hospitals, and the dogs did not show clinical signs of severe diar-

Fig. 1. Distribution of the locations (●) of samples collection in this study

The Qinghai Province located on the Qinghai Tibetan Plateau of China. The number represents the sampling site (sampling site name shown in Table 1)

Table 1. Prevalence and genotyping results for *Cryptosporidium* and *Giardia* detected in dogs in the Qinghai Tibetan Plateau Area

No.	Sampling sites	No. of samples analysed	*Cryptosporidium*		*Giardia*	
			Positive samples (%)	Species	Positive samples (%)	Assemblages
1	Gangcha sheep farm	10	1 (10.0)	*C. canis* (n=1)	0	
2	Hudong sheep farm	39	1 (2.56)	*C. parvum* (n=1)	1 (2.56)	B (n=1)
3	Sanjiaocheng sheep farm	23	1 (4.35)	*C. parvum* (n=1)	1 (4.35)	C (n=1)
4	Xining chengzhong dog farm	18	1 (5.56)	*C. andersoni* (n=1)	0	
5	Yushu sheep farm	16	1 (6.25)	*C. canis* (n=1)	1 (6.25)	B (n=1)
6	Beikeke sheep farm	8	0		0	
7	Xining chengbei dog farm	53	0		5 (9.43)	B (n=5)
8	Xining pet hospital	16	1 (6.25)	*C. parvum* (n=1)	0	
9	Tianjun sheep farm	5	0		0	
10	Qilian sheep farm	11	0		1 (9.09)	B (n=1)
11	Xining aiquan dog farm	18	0		6 (33.33)	D (n=3) C (n=2) B (n=1)
	Total	**217**	**6 (2.76)**		**15 (6.91)**	

rhoea. On sheep farms, dogs share typical habitats with humans and livestock on the grassland, and their functions are shepherding, guarding, protection and companionship. Both the humans (herders) and the animals (yaks, cattle and sheep) all possibly come into contact with these dog faeces. Dog farms are used for breeding, training, foster care, leisure, and training, and on them dogs chase and have contact with each other. Thus, the living environment may be contaminated with faeces. The pet hospitals provide medical services for pets, including dogs and cats. Sometimes, pet dog faeces will be collected for clinical examination and diagnosis. Therefore, most of these dogs were considered as pets and they would be in very close contact with humans.

Faecal specimen collection. A total of 217 dog faecal samples were collected from sheep farms, dog farms and pet hospitals (Table 1). Each individual faecal sample was kept in 2.5% potassium dichromate and then sent to the laboratory for further analysis. Within a month in the lab, the samples were washed several times with distilled water, then the total genomic DNA was extracted from each faecal sample with the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's instructions, with the addition of 10 freeze-thaw cycles.

Molecular detection of Cryptosporidium. Cryptosporidium PCR: a two-step nested-PCR technique was performed to amplify the fragment of the 18S rRNA gene to detect *Cryptosporidium* oocysts genomic DNA. The expected lengths obtained after primary amplification employed the primers 18SiCF2 and 18SiCR2, and the product was about 763 bp; the secondary amplification employed the primers 18SiCF1 and 18SiCR1, correspondingly generating a 587 bp product (RYAN et al., 2003); For more information, please see the supplementary Table 1.

Molecular detection of G. duodenalis. G. duodenalis PCR: for molecular detection of *G. duodenalis*, nested PCR targeting the β-giardin gene locus (CACCIO et al., 2002; LALLE et al., 2005) of *G. duodenalis* was carried out, as previously

Supplementary Table 1. PCR primers and cycling protocols to amplify target gene sequences from *Cryptosporidium* spp. and *Giardia duodenalis*

Parasite	PCR target	Size (bp)	Primer sequence (5′---3′)	Cycling protocol	Reference
Cryptosporidium spp.	18S rRNA gene	763	18SiCF2: GACATATCATTCAAGTTTCTGACC 18SiCR2: CTGAAGGAGTAAGGAACAACC	Both PCRs were performed in standard mixtures of 50 µl containing 4 µl primer mixtures (10 µM of each primer), 2 µl dNTP Mix (10 mM of each d NTP), 5 µl 10×PCR Buffer containing 1.5 mM MgCl2 (Qiagen), 3 µl 3 mM MgCl2 (Qiagen), 0.5 µl 5 U HotStar Taq DNA Polymerase (Qiagen), 3 µl bovine serum albumin (BSA; acetylated, 10 mg/mL) (Promega), 2.5 µl DNA, and 30 µl PCR-Grade water.	(RYAN et al., 2003)
		587	18SiCF1: CCTATCAGCTTTAGACGGTAGG 18SiCR1: TCTAAGAATTTCACCTCTGACTG	For primary PCR, the amplification reactions were run according to the following PCR program: an initial heat-activation step at 95°C for 15 min; 35 cycles of 94°C for 35 s, 58°C for 35 s, and 72°C for 50 s; then 72°C for 10 min and a final hold at 4°C. For secondary PCR, each reaction was prepared as for primary PCR, but 18SiCF1/R1 primers were used and the following PCR program was run: 95°C, 15 min; 35 cycles of 94°C for 30 s, 58°C for 30 s, and 7 °C for 30 s; then 72°C for 10 min and a final hold at 4°C.	
Giardia duodenalis	β-giardin gene (*bg*)	753	G7: AAGCCCGACGACCTCACCCGCAGTGC G759: GAGGCCGCCCTGGATCTTCGAGACGAC	Both PCRs were performed in standard mixtures of 50 µl containing 4 µl primer mixtures (10 µM of each primer), 2 µl dNTP Mix (10 mM of each d NTP), 5 µl 10×PCR Buffer containing 1.5 mM MgCl2 (Qiagen), 3 µl 3 mM MgCl2 (Qiagen), 0.5 µl 5 U HotStar Taq DNA Polymerase (Qiagen), 3 µl bovine serum albumin (BSA; acetylated, 10 mg/mL) (Promega), 2.5 µl DNA, and 30 µl PCR-Grade water.	(CCIÒ et al., 2002)
		515	G2F: GAACGAACGAGATCGAGGTCCG G2R: CTCGACGAGCTTCGTGTT	For primary PCR, the amplification reactions were run according to the following PCR program: an initial heat-activation step at 95°C for 15 min; 35 cycles of 94°C for 35 s, 50°C for 35 s, and 72°C for 60 s; then 72°C for 10 min and a final hold at 4°C. For secondary PCR, each reaction was prepared as for primary PCR, but G2F/G2R primers were used and the following PCR program was run: 95°C, 15 min; 35 cycles of 94°C for 35 s, 55°C for 35 s, and 72°C for 45 s; then 72°C for 10 min and a final hold at 4°C.	

described (Supplementary Table 1). The first and secondary amplifications employed the primers G759 and G7 (product: 753 bp), G2F and G2R (product: 515 bp), separately. Positive and negative controls were included in each amplification reaction. Genomic DNA from *Cryptosporidium* oocysts and *G. duodenalis* cysts was used as a positive control, and distilled water was used as a negative control for PCR. The amplified PCR products were visualized using a WD-9413B gel imaging analysis system (Beijing Liuyi Biotechnology, China) following electrophoresis on a 1.5% agarose gel, stained with nucleic acid electrophoresis dye (GelStain, TransGen Biotech Co., Ltd., China).

Sequencing and phylogenetic analysis. The positive PCR products were purified and sequenced with forward and reverse inner primers by SUZHOU GENEWIZ Company (Suzhou, China). To confirm their genotypes, the sequences were processed by Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/), and using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), aligning with the reference sequences in GenBank. Phylogenetic analysis of *Cryptosporidium* and *G. duodenalis* was achieved by the neighbour-joining (NJ) method, which was calculated by the Jukes-Cantor model with 2000 bootstrap replicates.

Results

A total of 217 dog faecal samples were collected from different locations in the QTPA of China (Fig.1 and Table 1), and examined to determine the prevalence of *Cryptosporidium* and *G. duodenalis* by PCR and sequencing analysis. Among them, six samples were found to be *Cryptosporidium*-positive, and 15 were found to be *G. duodenalis*-positive, as confirmed by PCR amplification of the 18S rRNA gene and β-giardin gene, with infection rates of 2.8% (6/217) and 6.9% (15/217), respectively. The results showed that *Cryptosporidium* spp. infections of dogs were present on several sheep farms (namely Gangcha, Hudong, Sanjiaochen and Yushu), on Xining Chengzhong dog farm and in Xining pet hospital. Likewise, dog *G. duodenalis* infections were found on sheep farms (including Hudong, Sanjiaochen, Yushu and Qilian) and two dog farms (Xining Chengbei and Xining Aiquan). No simultaneous coinfections of *Cryptosporidium* and *G. duodenalis* were detected at any sampling sites.

For *Cryptosporidium* spp., sequencing and phylogenetic analyses gave the following results: one *Cryptosporidium*-positive sample was detected in each of the sampling sites mentioned above, and these samples were identified as *C. parvum* (n=3), *C. canis* (n=2) and *C. andersoni* (n=1) (Table 1). The β-giardin gene sequencing and phylogenetic analyses of *G. duodenalis* were as follows: one *G. duodenalis*-positive faecal sample was detected on each of the sheep farm sampling sites, five on Xining Chengbei dog farm and six on Xining Aiquan dog farm, identified as *G. duodenalis* assemblages B, C and D (Table 1).

For *Cryptosporidium* spp., the partial sequences of the 18S rRNA locus revealed the presence of *Cryptosporidium* as *C. parvum*, *C. canis*, and *C. andersoni*, which all showed 99% similarity to the reference sequences in GenBank, with a query coverage of 100%. Concerning *G. duodenalis*, the partial sequences of the β-giardin gene determined the presence of *G. duodenalis* as *G. duodenalis* assemblage B, assemblage C and assemblage D, which showed 98% similarity to the β-giardin gene sequences, with a query coverage of 100%. The nucleotide sequences identified in our study were deposited in the GenBank database under the accession numbers MN038055 - MN038060 for *Cryptosporidium* and MN044595-MN044609 for *G. duodenalis*. The phylogenetic analysis employing the neighbour-joining (NJ) method indicated that representative sequences of the 18S rRNA gene and β-giardin gene from the *Cryptosporidium* and *G. duodenalis* species, generated in the present study, formed well-defined clusters with their respective reference sequences (Fig. 2 and 3).

Discussion

Dogs are of significance to humans in the QTPA. They are highly valued for companion-

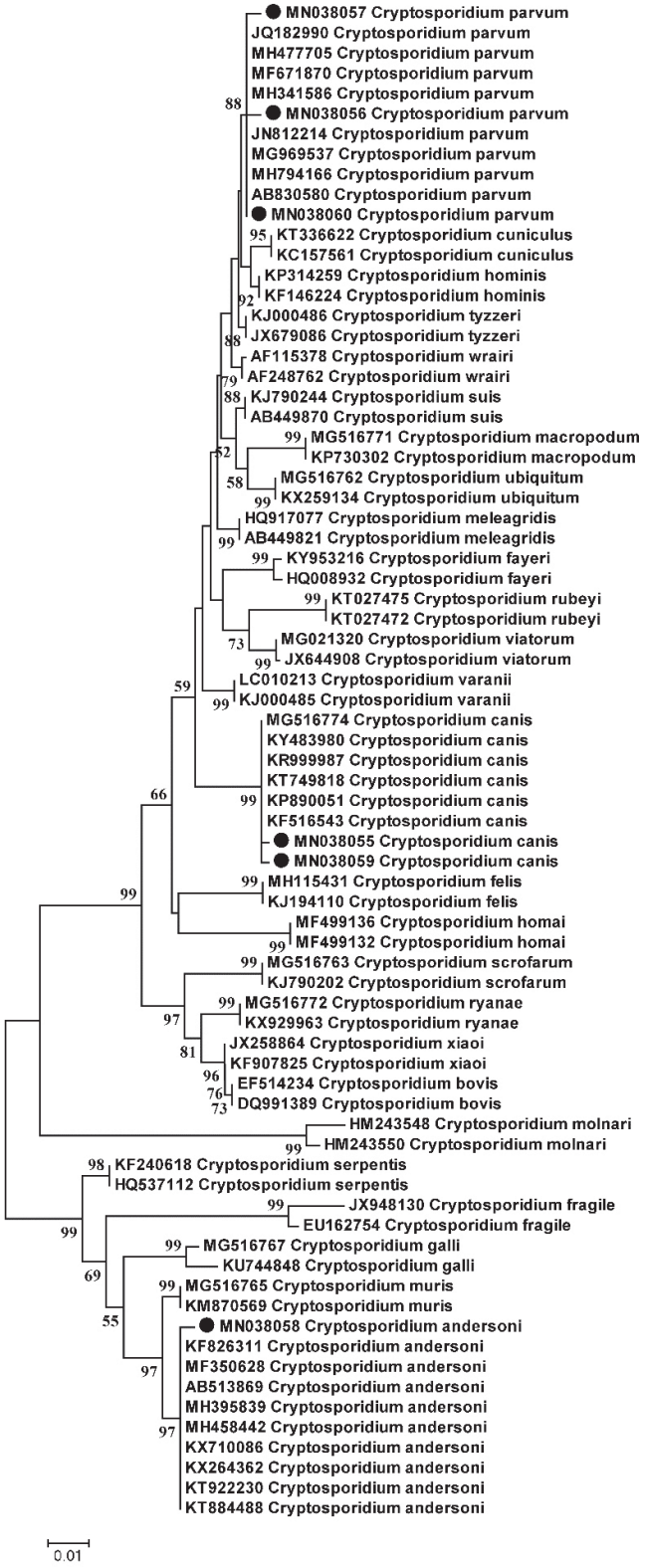

Fig. 2. Phylogenetic analysis of *Cryptosporidium* spp. based on sequences of the partial 18S ribosomal RNA gene

The black circles represent the positive samples and the species identified

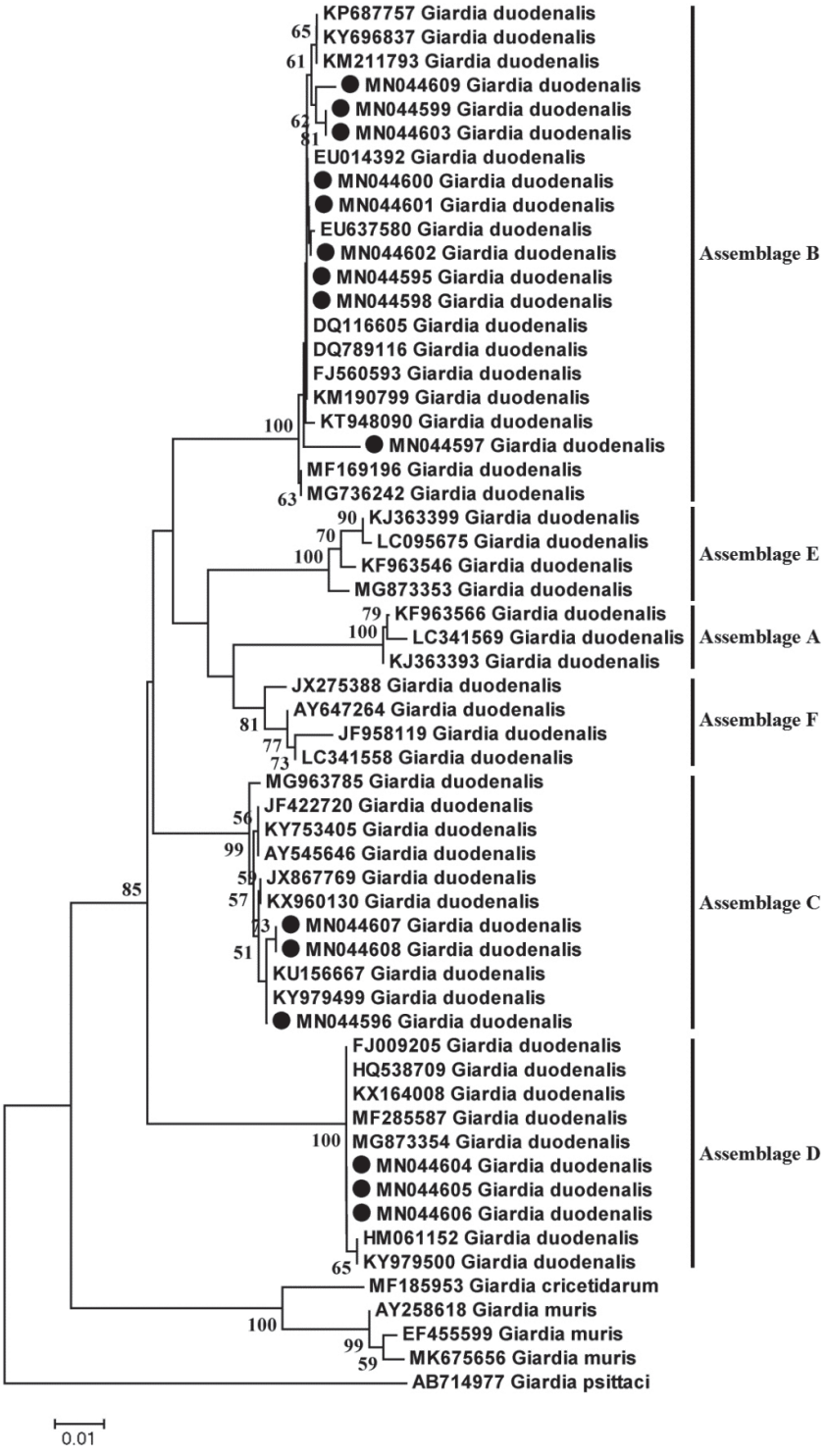

Fig. 3. Phylogenetic analysis of *Giardia* based on sequences of the partial β-giardin gene

The black circles represent the positive samples and the assemblages of *G. duodenalis* identified

ship, recreation, and protection of humans, but they are also reservoirs of pathogens for humans and animals. To assess the zoonotic potential of *Cryptosporidium* spp. and *G. duodenalis* from dogs, we conducted a molecular epidemiological survey of these pathogens in dogs in the QTPA. The present study represents the first investigation of the *Cryptosporidium* spp. and *G. duodenalis* infection prevalence rates and genotypes/species/assemblages in dogs in the QTPA of China.

The global prevalence of *Cryptosporidium* spp. and *G. duodenalis* infections in dogs in China varies depending on the geographic area and population tested (LI et al., 2013; JIAN et al., 2014; LI et al., 2015; ZHANG et al., 2016b; LIU et al., 2017; PAN et al., 2018; YANG et al., 2018; YU et al., 2018). In the present study, the overall *Cryptosporidium* spp. and *G. duodenalis* prevalence was 2.8% and 6.9%, determined by PCR detection and sequence identification, respectively. The prevalence of *Cryptosporidium* spp. in the QTPA dogs is consistent with other reports in China: 4.9% in companion dogs (YU et al., 2018), 1.6% in pet dogs (GU et al., 2015), 2.2% in dogs mainly from pet hospitals and pet markets, but also some stray dogs (LI et al., 2015). A higher occurrence of these pathogens was also observed in the following studies: 10.5% in raccoon dogs (ZHANG et al., 2016a), and 8.0 % in companion dogs (XU et al., 2016). On the other hand, the prevalence of *G. duodenalis* was lower than in previous studies carried out in other places, that is: 6.0 % in companion dogs (XU et al., 2016), 3.2% in pet dogs (GU et al., 2015), 4.5% in pet dogs (LI et al., 2015), 3.4% in pet dogs (GU et al., 2015), 7.2 % in farmed raccoon dogs (XU et al., 2016), and 6.7% in pet dogs (LI et al., 2015). Some other studies reported higher prevalences: 12.8% in companion dogs (YU et al., 2018), 11% in stray dogs (PAN et al., 2018), 26.2% in companion dogs (XU et al., 2016), 14.3% in pet and stray dogs (QI et al., 2016a), 15.2% in owned and stray dogs (YANG et al., 2015), 13.2% in police and farm dogs (LI et al., 2013), and 11% in pet dogs (LI et al., 2012).

In this study, the *Cryptosporidium* detection rates were lower, between 2.6 and 10%, in different regions, for *G. duodenalis* infections, the value of 2.6-9.4% was consistent with the prevalence data of *Cryptosporidium* except for one location, Xining Aiquan dog farm, which showed a higher prevalence (33.3%) than the other areas in this study. Many factors influence the prevalence of parasitic pathogens, such as the dogs' age group, the geographic area of origin, and the sampling time. Additionally, low starting quantities of parasite genomic DNA would negatively affect the detection sensitivity of the PCR protocols, particularly when the PCR target gene is a single-copy gene such as β-giardin used in this study.

In agreement with most previous related studies, we also identified *C. canis* as a host-specific species in dogs. Unexpectedly, *C. andersoni* was detected in dogs on the Xining Chengzhong dog farm. It had been identified as the predominant species responsible for cattle infections (QI et al., 2016b), but also as the predominant *Cryptosporidium* species in humans in China (JIANG et al., 2014). In the QTPA of China, *C. andersoni* was also detected in slaughterhouses, sewage, and river waters (MA et al., 2019), as well as in sheep, yaks and cattle (LI et al., 2016; ZHANG et al., 2018), which demonstrated the widespread distribution of *C. andersoni* in the environment generally in this region. Although reports of dog and human infections with *C. andersoni* are rare (ROSANOWSKI et al., 2018), one study suggested its role in clinical disease in China (JIANG et al., 2014). It should be noted that dogs are occasional transport host, and not a patent new host for *C. andersoni*; so further studies in this regard are required. Another outcome was the detection of *C. parvum* in three of the dog isolates and genotypes. *C. parvum* not only infects humans, but has also been responsible for diarrhoea in neonate animals, calves, and lambs. *C. parvum* was also identified in street dogs in Nigeria (AYINMODE et al., 2018), in dogs in Brazil (ALVES et al., 2018), in household dogs in Egypt (GHARIEB et al., 2018), in abandoned dogs in Great Britain (ROSANOWSKI et al., 2018), in Iranian dogs (RANJBAR et al., 2018), in dogs in Northern Italy (SIMONATO et al., 2017), in dogs in Chiang Mai, Thailand (TANGTRONGSUP et al., 2017) and in dogs in Costa Rica (SCORZA et al., 2011).

With respect to *G. duodenalis*, the molecular results revealed that the dogs were infected by host-specific *G. duodenalis* assemblages C/D (6 isolates), whereas potential human-pathogenic B assemblage was also found. These *G. duodenalis* assemblages have also been detected in dogs in other regions of China, for instance, assemblages A, C and D in stray dogs in Guangdong (PAN et al., 2018), C and D in companion dogs in Beijing (YU et al., 2018), C and D in dogs in Sichuan Province (ZHANG et al., 2017), C and D in pet dogs in Shanghai (LIU et al., 2017), C and D in pet and stray dogs in Henan Province (QI et al., 2016a), and C in dogs in Heilongjiang Province (LI et al., 2015).

The dog faecal samples were collected from sheep farms, dog farms and pet hospitals. Since dogs are involved in shepherding, guarding, protection and companionship of humans and animals, they may act as reservoirs for transmission of *Cryptosporidium* and *G. duodenalis* to humans and livestock animals.

Conclusions

This is the first study on *Cryptosporidium* species and *G. duodenalis* simultaneously detected in dogs in the QTPA. *C. parvum*, *C. canis*, *C. andersoni* and *G. duodenalis* assemblages B, C and D were found, respectively. Dogs are potential reservoirs for human and animal infections of these pathogens. Further studies are needed to extend investigations of *Cryptosporidium* and *G. duodenalis* species/genotypes/assemblages from humans (households and owners) and animals (yaks, cattle and sheep) living in the same environments and sharing the same water supplies.

Ethical approval

Written informed consent was obtained from all dogs' owners, and no dogs were harmed during the experimental process. Before the initiation of the experiments, the protocol of the current study was reviewed and approved by the Institutional Animal Care and Use Committee of the Qinghai Academy of Animal Sciences and Veterinary Medicine.

Financial support statement

The research was supported by the National Natural Science Foundation of China (NO. 32060804), the National Foreign Experts Program of China (Grant No. G2022043003L), the Transformation of Scientific and Technological Achievements of Qinghai Province (2021-SF-146) and the funding support from the One Thousand Talents Plan of the Chinese Government (NO. WQ2013630172).

Authorships contribution statement

Yingna Jian and Xueyong Zhang contributed equally to this work. Y.J., X.Z., and P.K. designed, conceived the study, and wrote the manuscript. Y.J., X.Z., L.M., G.H.W., and G.P.W. carried out the experiments and analysed the data. G.H.W., Q.C., G.P.W. and X.L. contributed to the collection of the faecal samples. Y.J., X.Z., and P.K. contributed to the discussion of results and the final version of the manuscript. All authors have approved the submission and publication of this manuscript.

Declaration of competing of interest

The authors declare no conflict of interests.

Acknowledgments

We would like to acknowledge Catherine O' Dowd Phanis, University of Nicosia Medical School, Nicosia, Cyprus for time devoted to improving the linguistic quality of this manuscript.

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Received: 26 July 2023
Accepted: 24 June 2024
Online publication: 31 December 2024

JIAN, Y., X. ZHANG1, L. MA, G. WANG, Q. CAI, G. WANG1, X. LI, P. KARANIS1: Molekularna detekcija parazita *Cryptosporidium* i *Giardia duodenalis* u uzorcima fecesa pasa s tibetanske Qinghai visoravni u Kini. Vet. arhiv 95, 197-210, 2025.

SAŽETAK

Psi su prirodni rezervoari velikog broja zoonotskih patogena, uključujući intestinalne parazite *Cryptosporidium* spp. i *Giardia duodenalis*, najvažnije protozoalne vrste koje uzrokuju gastrointestinalne bolesti diljem svijeta. Kako bi se ustanovila raširenost i molekularna raznolikost parazita *Cryptosporidium* i *G. duodenalis*, provedeno je epidemiološko istraživanje u području tibetanske Qinghai visoravni (QTPA) u sjevernoj Kini. Prikupljeno je ukupno 217 uzoraka fecesa pasa s farmi ovaca, iz uzgajališta pasa i iz bolnica za kućne ljubimce. Izolirane vrste odnosno genotipovi parazita *Cryptosporidium* i *G. duodenalis* identificirani su PCR-om usmjerenim na dio gena za ribosomsku RNA 18S za *Cryptosporidium* i gena β-giardin za *G. duodenalis*. Rezultati invazije parazitima *Cryptosporidium* spp. i *G. duodenalis* u pasa pokazali su ukupnu prevalenciju od 2,8% (6/217) za *Cryptosporidium* spp. i 6,9% (15/217) za *G. duodenalis*. Nije uočena istodobna invazija ovim dvama parazitima. Sekvenciranje PCR-om potvrdilo je nalaz *C. parvum* (n=3), *C. canis* (n=2), *C. andersoni* (n=1) i *G. duodenalis* podtipova B (n=9), C (n=3) i D (n=3). Rezultati su pokazali da su psi na istraživanom području često izloženi parazitima *Cryptosporidium* spp. i *G. duodenalis*. Navedeno potvrđuje da bi psi mogli biti potencijalan izvor invazije parazitima *Cryptosporidium* spp. i *G. duodenalis* za ljude i druge vrste životinja.

Ključne riječi: *Cryptosporidium*; *Giardia duodenalis;* psi; prevalencija; molekularna detekcija; tibetanska visoravan