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PROTOZOOLOGY - ORIGINAL PAPER

Occurrence of *Cryptosporidium* and *Giardia* in wild birds from Qinghai Lake on the Qinghai-Tibetan Plateau, China

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Abstract

Cryptosporidium and *Giardia* are important intestinal zoonotic pathogens that can infect various hosts and cause diarrhoeal diseases. There are few reports of the epidemiological prevalence and molecular characterization of *Cryptosporidium* and *Giardia* in wild birds around Qinghai Lake and in the surrounding areas on the Qinghai-Tibetan Plateau, Northwest China. Therefore, the aim of this study was to determine the *Cryptosporidium* spp. and *Giardia duodenalis* genotypes and their epidemiological prevalence in wild birds by PCR amplification. To our knowledge, this is the first report of a variety of *Cryptosporidium* spp. and *G. duodenalis* infections in wild birds from that area, with overall prevalence rates of 8.98% (61/679) and 3.39% (23/679), respectively. Furthermore, PCR sequencing confirmed the presence of *Cryptosporidium baileyi* ($n = 3$), *Cryptosporidium parvum* ($n = 58$), and *G. duodenalis* assemblage B ($n = 19$) and E ($n = 4$) in wild birds from the areas around Qinghai Lake. The results of the present study demonstrated the wide distribution of *Cryptosporidium* and *Giardia* among wild birds, which has potential public health significance. Moreover, the study findings also provided useful molecular epidemiological data for monitoring and investigating the two parasitic protozoa in wild animals and surrounding environments.

Keywords *Cryptosporidium* · *Giardia* · Wild birds · Prevalence · Molecular characterization · Qinghai Lake (China)

Introduction

Cryptosporidium spp. and *Giardia* spp. are common zoonotic enteric protozoan parasites that can infect a wide range of vertebrate hosts, including humans, mammals, and domestic and wild animals worldwide (Feng et al. 2018; Heyworth 2016; Plutzer et al. 2010; Ryan et al. 2014). Currently, birds are mainly infected by four avian *Cryptosporidium* species based on biological characteristics and genetic differences: *Cryptosporidium meleagridis*, *Cryptosporidium baileyi*, *Cryptosporidium avium*, and *Cryptosporidium galli* (Plutzer and Karanis 2009; Wang et al. 2019). Meanwhile, other *Cryptosporidium* species (*Cryptosporidium andersoni*, *Cryptosporidium parvum*, *Cryptosporidium hominis*, *Cryptosporidium muris*) and genotypes (*Cryptosporidium* goose genotypes (I–IV), a *Cryptosporidium* duck genotype, and *Cryptosporidium* avian genotypes (I–IV)) have also been reported in birds in previous studies (Cui et al. 2018; Nakamura and Meireles 2015; Ryan 2010). *C. meleagridis* is considered the third most prevalent species known to infect humans after *C. hominis* and *C. parvum* (Braima et al. 2019; Una et al. 2001). Based on multiple gene loci analysis, Xiao et al. (2002) have suggested that mammals were the original hosts of *C. meleagridis*. In general, many of the *Cryptosporidium* species and genotypes have a host specificity and are not usually considered a public health concern. However, some hosts carry zoonotic species, which contribute to cross-infection between host species (Braima et al. 2019). Furthermore, some

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genetically distinct avian *Cryptosporidium* genotypes/species, such as *Cryptosporidium* avian genotype I, *C. avium* and *C. proventriculi* in Psittaciform birds (Holubova et al. 2019), *C. parvum* in falcons (Azmanis et al. 2018), *C. parvum* and *Cryptosporidium* genotype BrPR1 in free-range chickens (Ewald et al. 2017), and *Cryptosporidium* duck genotype in a mandarin duck (*Aix galericulata*) (da Cunha et al. 2017), have recently been reported. Likewise, two species of *Giardia* have been recognized in avian hosts based on the morphology of trophozoites and cysts: *G. ardeae* and *G. psittaci* (Ryan and Caccio 2013). Other species/assemblages have been described from bird hosts; for instance, *G. duodenalis* assemblage A has been detected in a toco toucan (*Ramphastos toco*) (da Cunha et al. 2017) and *G. duodenalis* assemblage B, assemblage D, and assemblage F in wild birds from northwest Spain (Reboredo-Fernandez et al. 2015). Assemblages A and B are considered to be zoonotic and pathogenic to humans (Ryan and Caccio 2013).

Previous studies have confirmed that *Cryptosporidium* and *Giardia* are prevalent in livestock and wild animals (Itagaki et al. 2005; Jian et al. 2018; Oates et al. 2012; Wang et al. 2017, 2018a; Zhang et al. 2018a; Ziegler et al. 2007). Moreover, these two parasitic pathogens have attracted increasing attention, resulting in a series of epidemiological investigations focusing on public and veterinary health. Recently, *Cryptosporidium* and *Giardia* have been considered emerging pathogens in poultry and wild bird groups and are becoming prevalent parasites affecting domestic, caged, ornamental, companion, and wild birds. Infection of economic poultry (laying and meat chickens, ducks, and geese) with these two parasites may lead to extensive economic losses (Batz et al. 2012; Holubova et al. 2018; Majewska et al. 2009). Wang et al. (2012) found a 13.1% prevalence of *Cryptosporidium* from 47 quail farms in Henan, China, where *C. baileyi* was found in the majority of the positive samples. *C. baileyi* is generally associated with the respiratory form of cryptosporidiosis in birds and capable of infecting a variety of avian hosts. Most studies have focused on domestic animals (cattle, sheep, goat, yak, horse, chicken, and pig) of commercial interest (Hu et al. 2017; Li et al. 2016a; Majewska et al. 2009; Petersen et al. 2015; Qi et al. 2015, 2019; Squire et al. 2017; Wang et al. 2018a, 2018b; Zhong et al. 2018). McEvoy and Giddings (2009) have reported that while *C. parvum* was detected on a large turkey farm and post slaughter, *C. parvum* was not a significant reservoir for *Cryptosporidium* species. In comparison, relatively fewer studies involved wild birds infected with *Cryptosporidium* and *Giardia* (Cano et al. 2016; da Cunha et al. 2017; Majewska et al. 2009; Plutzer and Tomor 2009; Reboredo-Fernandez et al. 2015). Notably, various studies have identified and demonstrated the occurrence of the zoonotic species *C. parvum* in wild birds, suggesting that infected birds may play an important role in harbouring and disseminating this parasitic pathogen (Plutzer and Tomor 2009; Reboredo-Fernandez et al. 2015). For the zoonotic *G. duodenalis* assemblages, A and B have also been reported in birds (Cano et al. 2016; da Cunha et al. 2017; Plutzer and Tomor 2009).

Qinghai Lake is located in the north eastern part of the Qinghai-Tibetan Plateau (QTP), with an altitude of approximately 3200 m, covering an area of approximately 4500 km^2, and with a circumference of more than 360 km. The sources of water for the lake are from rivers, precipitation, and a spring at the bottom of the lake. The most important water sources are rivers, with more than 40 rivers that, including the Buha River, Shaliu River, Wuha Alam River, and Haage River, deposit into the lake. There are many more rivers on the southwest, northwest, and north coast, with large drainage areas and many tributaries. The environmental conditions and geographic location of Qinghai Lake make it a suitable habitat for wild birds; there are 220 species and more than 160,000 birds, including the bar-headed goose (*Anser indicus*), brown-headed gull (*Chroicocephalus brunnicephalus*), great cormorant (*Phalacrocorax carbo*), Crested duck (*Anas platyrhynchos domesticus*), ruddy shelduck (*Tadorna ferruginea*), common merganser (*Mergus merganser*), Chinese spot-billed duck (*Anas zonorhyncha*), Northern pintail (*Anas acuta*), whooper swan (*Cygnus cygnus*), and black-necked crane (*Grus nigricollis*), reported from the area. Further, a small study has identified 5/148 (3.38% prevalence) positive samples for *C. baileyi* genotypes in ruddy shelducks from the Qinghai Lake (Amer et al. 2010). Qinghai Lake has become a major breeding site for migratory birds flying to Australia, India, Siberia, and Southeast Asia via the Central Asian-Indian flyway and the East Asian-Australian flyway (Dong et al. 2017).

However, very few studies on the presence of *Cryptosporidium* and *Giardia* in wild birds have been performed in this area. The aim of this study was to determine the prevalence and molecular characterization of *Cryptosporidium* and *Giardia* species/genotypes in faecal samples from wild birds around Qinghai Lake on the QTP of China.

Materials and methods

Study sites

The faecal samples analysed in the present study were collected from wild birds at different locations around Qinghai Lake on the QTP of China (see Fig. 1). The sampling sites were located in the northern (Quanji River estuary, Fairy Bay, Shaliu River estuary, Hadatan wetlands, Naren wetlands and Sheng River estuary) and western (bird rescue centre, Egg Island, Bird Island Park and Cormorant Island) parts of Qinghai Lake, including the river estuaries, wetlands, and islands. These areas are all breeding sites and suitable habitats for wild birds.

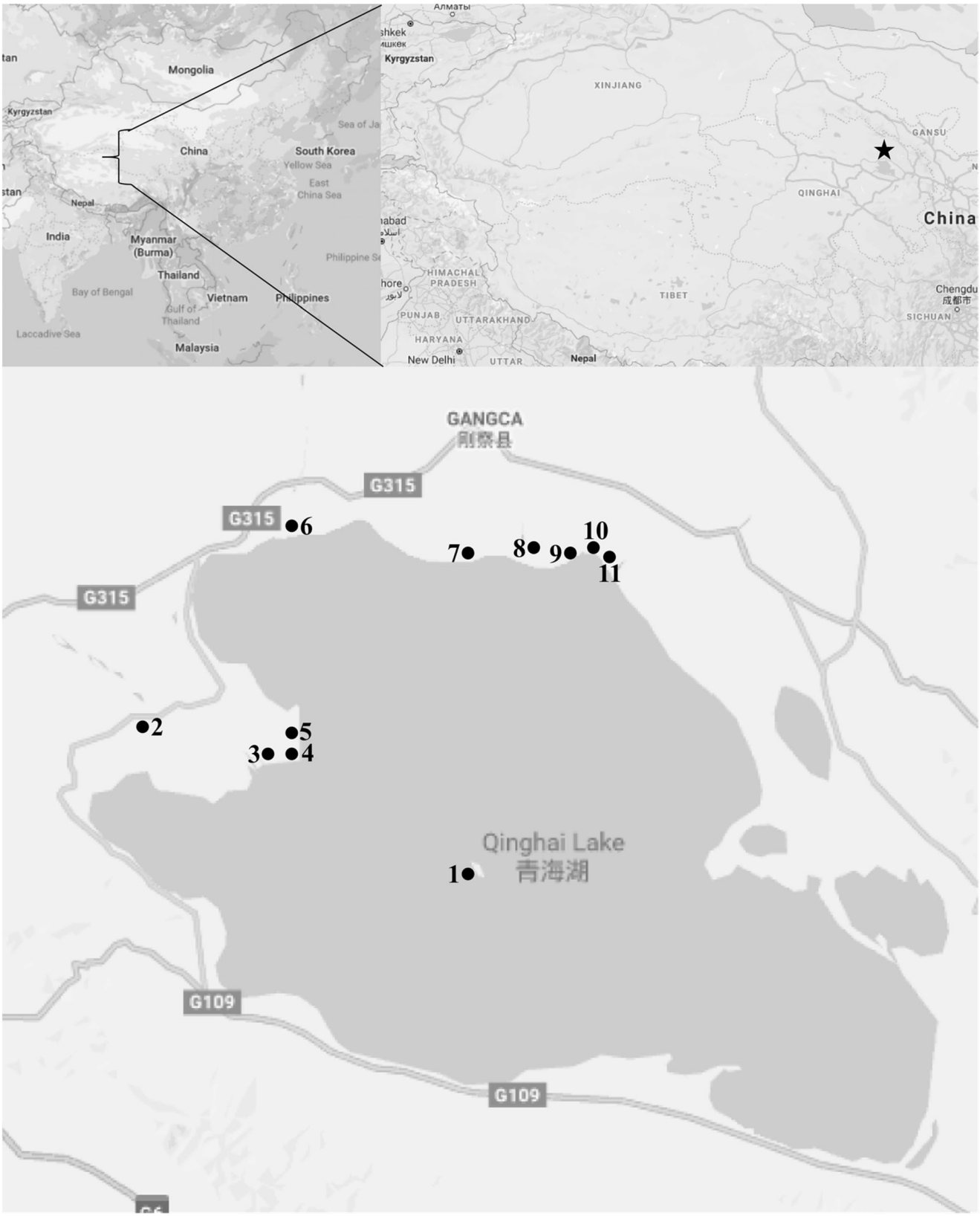

Fig. 1 Distribution of the sample collection locations (●) in this study. Qinghai Lake is located on the Qinghai-Tibetan Plateau in China. The five-pointed star (★) represents Qinghai Lake, and the number represents the sampling site (sampling site names: 1: Haixinshan Island, 2: Bird Rescue Center, 3: Egg Island, 4: Bird Island Park, 5: Cormorant Island, 6: Quanji River Estuary, 7: Fairy Bay, 8: Shaliu River Estuary, 9: Hadatan Wetlands, 10: Naren Wetlands, 11: Sheng River Estuary)

Specimen collection

A total of 679 individual wild bird faecal samples were collected from the ground around Qinghai Lake in 2016 and 2018. Fresh faecal samples were preferentially chosen when available. The samples were collected on site in cooperation with the staff members of the Qinghai Lake National Nature Reserve Administration, and they were fresh at the time of collection. Upon observing groups of birds, the observers walked towards them and collected the faeces. The main bird species were brown-headed gull, bar-headed goose, great cormorant, and great black-headed gull (*Larus ichthyaetus*). Each individual fresh faecal sample was placed in a sterile polystyrene tube (50-ml centrifuge tube) with records of the date, location, and identification number. The samples were kept in 2.5% potassium dichromate and transferred in isothermal boxes to the laboratory in Xining where they were stored at − 20 °C until DNA extraction. The total genomic DNA was extracted from each faecal sample with a QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's instructions, with the addition of 10 freeze-thaw cycles.

Molecular characterization of *Cryptosporidium* and *Giardia* spp.

A two-step nested-PCR technique was performed to amplify a fragment of the 18S rRNA gene to detect *Cryptosporidium* oocysts. The expected length fragments were obtained after primary amplification with the primers 18SiCF2: 5′-GACA TATCA TTCAAGTTTCTGACC-3′ and 18SiCR2: 5′-CTGAAGGAGTAAGGAACAACC-3′; the product was approximately 763 bp. The secondary amplification was conducted with the primers 18SiCF1: 5′-CCTATCAGCTTTAG ACGGTAGG-3′ and 18SiCR1: 5′-TCTAAGAATTTCAC CTCTGACTG-3′, generating a corresponding 587-bp product (Ryan et al. 2003). Both PCRs were performed with standard mixtures of 50 μl containing 4 μl primer mixtures (10 μM of each primer), 2 μl dNTP mix (10 mM of each dNTP), 5 μl 10 × PCR buffer containing 1.5 mM $MgCl_2$ (Qiagen), 3 μl 3 mM $MgCl_2$ (Qiagen), 0.5 μl 5 U HotStart 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. For the primary PCRs, the amplification reactions were run according to the following PCR programme: 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 the secondary PCRs, each reaction was prepared as for the primary PCR, but 18SiCF1/R1 primers were used, and the following PCR programme was run: 95 °C for 15 min; 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s; then 72 °C for 10 min and a final hold at 4 °C. For the molecular detection of *Giardia*, a nested PCR was also performed to amplify a 292-bp fragment of the *Giardia* 18S rRNA gene locus according to Appelbee et al. (2003) to detect *Giardia* cysts. The protocol used to detect *Cryptosporidium*, except the primers and the PCR programme, was different as follows: the primary primers used were Gia2029F: 5′-AAG TGT GGT GCA GAC GGA CTC-3′ and Gia2150R: 5′-CTG CTG CCG TCC TTG GAT GT-3′; the secondary primers used were RH11 5′-CAT CCG GTC GAT CCT GCC-3′ and RH4 5′-AGT CGA ACC CTG ATT CTC CGC CAG G-3′; and the annealing temperatures were 55 °C and 59 °C, respectively. A positive control and negative control were included in each amplification. The amplified PCR products were analysed using 1.5% agarose gel containing ethidium bromide (0.6 mg/mL) and were observed under UV light.

Sequencing and phylogenetic analysis

The positive PCR products were sequenced by SUZHOU GENEWIZ Company (Suzhou, China). To confirm their genotypes, the sequences were analysed by Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) alignment with reference sequences in GenBank. The phylogenetic analyses of *Cryptosporidium* and *Giardia* were performed with the neighbour-joining (NJ) method, which was calculated by the Jukes-Cantor model with 2000 bootstrap replicates.

Results

In this study, a total of 679 fresh wild bird faecal samples were collected from different locations around Qinghai Lake on the QTP of China (Fig. 1) from 2016 to 2018 to study the prevalence of *Cryptosporidium* and *Giardia* by PCR and sequencing analysis. Among the samples, 61 specimens were *Cryptosporidium*-positive, and 23 were *Giardia*-positive, as confirmed by PCR amplification of the rRNA genes, with infection rates of 8.98% (61/679) and 3.39% (23/679), respectively. In detail, the results showed that *Cryptosporidium* spp. infection in wild birds was prevalent at the bird rescue centre, Egg Island, Quanji River estuary, and Fairy Bay. Notably, *Giardia* infection in wild birds was found in more places: the bird rescue centre, Egg Island, Fairy Bay, Shaliu River estuary, the Hadatan wetlands, and the Naren wetlands. The numbers of co-infections of *Cryptosporidium* and *Giardia* were three for Egg Island (*C. parvum*, $n = 3$, *G. duodenalis* assemblage B, $n = 3$) and one for Fairy Bay (*C. parvum*, $n = 1$, *G. duodenalis* assemblage B, $n = 1$). For *Cryptosporidium* spp., sequencing and phylogenetic analyses identified the following: fifteen *Cryptosporidium*-positive faecal samples were detected from the bird rescue centre (15/153, 9.80%), 41 from Egg Island (41/311, 13.18%), one from the Quanji River

estuary (1/17, 5.88%), and four from Fairy Bay (4/95, 4.21%); the species were identified as *C. parvum* ($n = 58$) and *C. baileyi* ($n = 3$). The sequencing and phylogenetic analyses of *Giardia* were as follows: five *Giardia*-positive faecal samples were detected from the bird rescue centre (5/153, 3.27%, assemblage B), 12 from Egg Island (12/311, 3.86%, assemblages E ($n = 1$) and B ($n = 11$), three from Fairy Bay (3/95, 3.16%, assemblage B), and one each from the Shaliu River estuary (1/21, 4.76%), Hadatan wetlands (1/10, 10.00%), and Naren wetlands (1/14, 7.14%); all identified as assemblage E.

For *Cryptosporidium* spp., the partial sequences of the 18S rRNA locus identified the species *C. baileyi*, with 97% similarity with *C. baileyi* (MH062741/2, MF498750, KY448455/6/8, KY352487/8/9) with a query coverage of 99%. For *C. parvum*, there was 100% similarity with *C. parvum* (MF589923, MH477699, MH074867, KY514066, KT948751, KP994663, KP730314, KP004203, KJ808693, KC886318, EU553550, EF175936, DQ833278, DQ656354, AJ853993/4, AJ849463, AF308600) with a query coverage of 100%. With respect to *Giardia* spp., the partial sequences of the 18S rRNA determined the presence of *G. duodenalis* assemblage E, which showed 100% similarity to assemblage E (MK573336/28, MG958618, KF843921, JF957620, KR048478-91) with a query coverage of 100%. Moreover, *G. duodenalis* assemblage B presented 100% similarity with assemblage B (MG018739, KY658186/7, JX972180, HQ616612) with a query coverage of 100%. The nucleotide sequences identified in our study were deposited in the GenBank database under the accession numbers MK992409-MK992469 for *Cryptosporidium* and MK993304-MK993326 for *Giardia*. The phylogenetic analyses employing the NJ method indicated that all 18S rRNA representative gene sequences of the *Cryptosporidium* and *Giardia* species identified in the present study formed well-defined clusters with their respective reference sequences (Figs. 2 and 3).

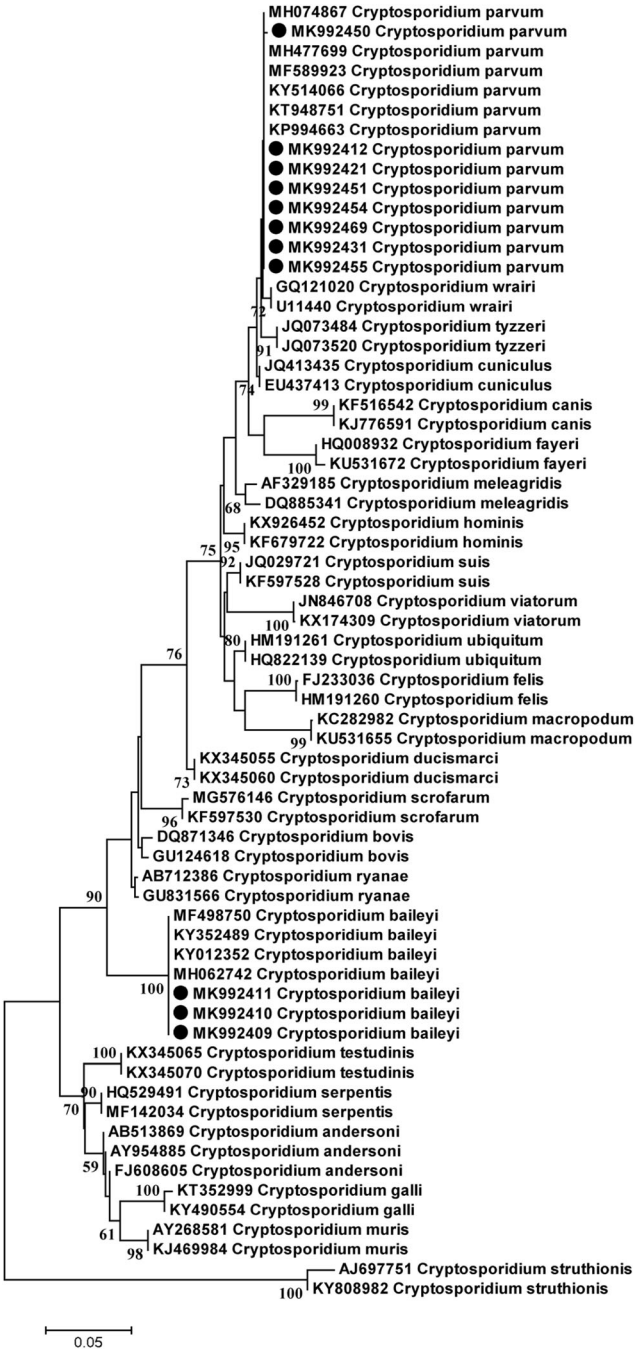

Fig. 2 Phylogenetic analysis of *Cryptosporidium* spp. based on sequences of the partial small subunit ribosomal RNA gene. Black circles represent the positive samples in this study

Discussion

This is the first large parasitological study involving the molecular characterization and epidemiological prevalence detection of *Cryptosporidium* and *Giardia* species in wild birds around Qinghai Lake on the QTP in China. For this location and the wild birds in this area, the main focus so far has been on avian influenza. Little is known about the occurrence of *Cryptosporidium* and/or *Giardia* in wild birds here, with only one study to date (Amer et al. 2010). Many researchers suggest to monitor migratory waterfowl as a model for potential source contamination for water supplies that extend to humans, farms, and wildlife (Rao et al. 2009). Most previous studies from other locations around the world have also focused on the transmission of these two parasitic pathogens in aquatic and migratory birds (Cano et al. 2016; Elmore et al. 2013; Plutzer and Tomor 2009).

The overall prevalence of *Cryptosporidium* in the samples was 8.98%, as determined by PCR analysis. Prevalence data from other studies on waterbirds (see Table 1) indicate a wide scope of infection rates, ranging from 0.5% in Canada geese (*Branta canadensis*) (Zhou et al. 2004) to 100% in ducks (da Cunha et al. 2017; Kuhn et al. 2002), the black-headed gull (*Chroicocephalus ridibundus*) (Medema 1999), great

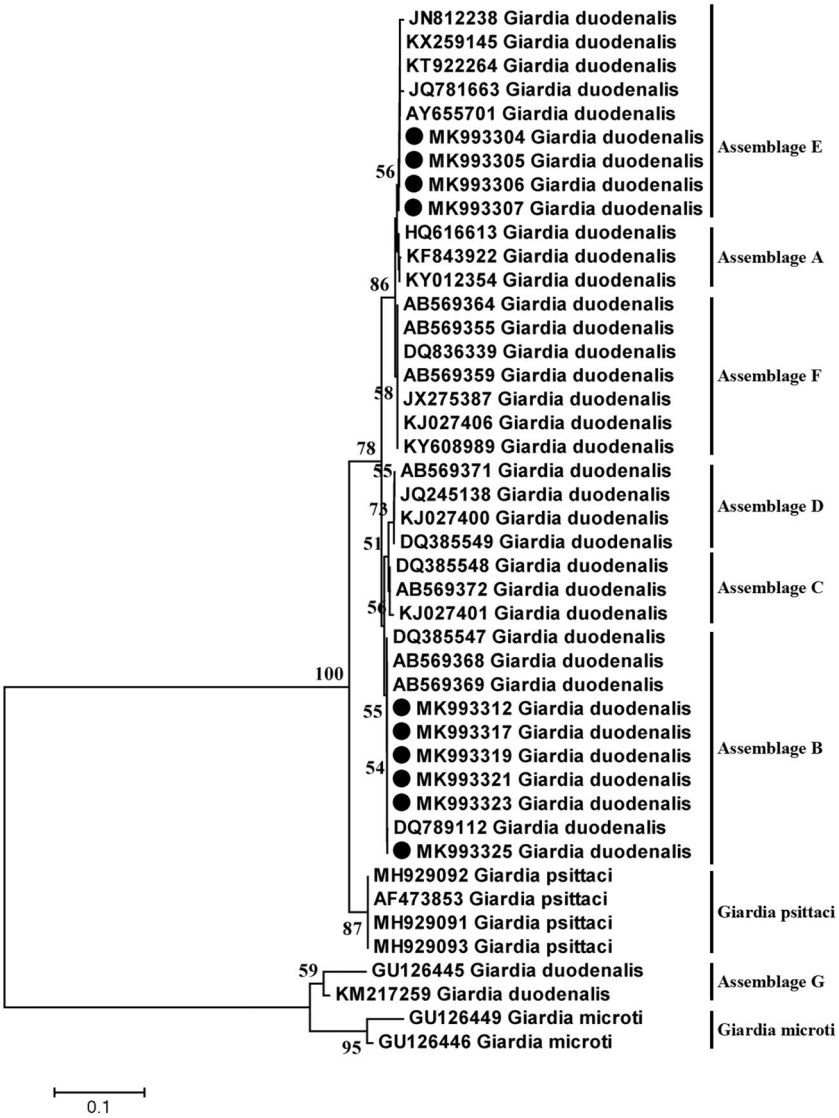

Fig. 3 Phylogenetic analysis of *G. duodenalis* based on sequences of the partial 16S rRNA gene. Black circles represent the positive samples in this study

cormorant (Plutzer and Tomor 2009), and hooded merganser (*Lophodytes cucullatus*) (Kuhn et al. 2002), although in all cases with 100% infection rates, sample size was small, with only one to three faecal samples analysed. Similar results to ours have been reported in various species, with infection rates of 2% and 3.4% in the Greylag goose (*Anser anser*) (Plutzer and Tomor 2009), 2.8% in the common merganser (Majewska et al. 2009), 3.38% in the ruddy shelduck (Amer et al. 2010), 0.5 to 6.8% in the Canada goose (Jellison et al. 2004; Zhou et al. 2004), 4.95% in the black-headed gull and European herring gull (*Larus argentatus)* (Smith et al. 1993), 7.7% in the White stork (*Ciconia ciconia*) (Reboredo-Fernandez et al. 2015), and 8% in the great cormorant (Rzymski et al. 2017). Moreover, infection with *Cryptosporidium* is found in a wide geographic range (Table 1). Most studies are from Europe, including the Czech Republic (Pavlasek 1993), Germany (Richter et al. 1994), Hungary (Plutzer and Tomor 2009), the Netherlands (Medema 1999), Poland (Majewska et al. 2009; Rzymski et al. 2017), Scotland (Smith et al. 1993), and Spain (Cano et al. 2016; Reboredo-Fernandez et al. 2015), and the Americas, with the USA (Jellison et al. 2004; Kassa et al. 2004; Kuhn et al. 2002; Zhou et al. 2004) and Brazil (Bomfim 2013; da Cunha et al. 2017; Nakamura et al. 2009). There are fewer studies from Asia, including China (Amer et al. 2010; Wang et al. 2010), Iran (Larki et al. 2018; Shemshadi et al. 2014, 2016), and Thailand (Koompapong et al. 2014) with one study from Australia (Ng et al. 2006). It is obvious that *Cryptosporidium* is a widespread parasite, widely dispersed among waterbirds and continents.

Additionally, *Cryptosporidium* has been found in domestic, captive and wild terrestrial avian hosts worldwide (Nakamura and Meireles 2015). Studies on domestic birds, for example, have reported prevalence rates of 0.82% in domestic pigeons in Guangdong Province, southern China (Li et al. 2015); 3.8% in free-ranging, captive, and domestic birds in western Poland (Majewska et al. 2009); 7% in carrier

Table 1 Species of waterbirds, origin, and species/genotypes of *Cryptosporidium* positives (in %) during the period 1990–today

Species name/common name	Geographic origin	Species or genotype	% positive for *Cryptosporidium* spp. (no. positive/no. sampled)	Reference
Aix galericulata (Mandarin duck)[a]	Minas Gerais, Brazil	Duck genotype	100 (1/1)	da Cunha et al. 2017
	Poland	*Cryptosporidium parvum*	33.3 (1/3)	Majewska et al. 2009
	Brazil	*Cryptosporidium* spp.	20 (1/5)	Nakamura et al. 2009
Anas americana (American wigeon)	Rio Grande, New Mexico, USA	*Cryptosporidium parvum*	100 (3/3)	Kuhn et al. 2002
Anas carolinensis (Green-winged teal)	Rio Grande, New Mexico, USA	*Cryptosporidium parvum*	50 (3/6)	Kuhn et al. 2002
Anas discors (Blue-winged teal)	Rio Grande, New Mexico, USA	*Cryptosporidium* spp.	50 (2/4)	Kuhn et al. 2002
Anas penelope (Eurasian wigeon)	Biesbosch Reservoirs, Netherlands	*Cryptosporidium* spp.	25 (1/4)	Medema 1999
Anas platyrhynchos (Mallard)	Lake Balaton, Hungary	*Cryptosporidium parvum*	18.1 (2/11)	Plutzer and Tomor 2009
	Galicia, Spain	*Cryptosporidium* spp.	50 (2/4)	Reboredo-Fernandez et al. 2015
	Rio Grande, New Mexico, USA	*Cryptosporidium parvum*	45 (23/51)	Kuhn et al. 2002
	China	*Cryptosporidium baileyi*	16.3 (92/564)	Wang et al. 2010
Anas platyrhynchos domesticus (Mallard)[b]	Gilan Province, Iran	*Cryptosporidium baileyi*	16.6 (5/30)	Shemshadi et al. 2016
	Rio de Janeiro, Brazil	*Cryptosporidium baileyi*	76.6 (46/60)	Bomfim 2013
	Germany	*Cryptosporidium* spp.	57 (73/128) *	Richter et al. 1994
	Ahvaz, Iran	*Cryptosporidium* spp.	50 (11/41)	Larki et al. 2018
	Hungary	*Cryptosporidium baileyi (?)*	10.3 (3/29) **	Plutzer and Tomor 2009
Anas sp. (Ducks)[a]	Brazil	*Cryptosporidium* spp.	20 (1/5)	Nakamura et al. 2009
Anas strepera (Gadwall)	Biesbosch Reservoirs, Netherlands	*Cryptosporidium* spp.	13 (1/8)	Medema 1999
Anser anser (Greylag goose)	Lake Balaton, Hungary	*Cryptosporidium* spp.	2 (1/48)	Plutzer and Tomor 2009
Anser anser domesticus (Greylag goose)[b]	Hungary	*Cryptosporidium baileyi*	3.4 (1/29) **	Plutzer and Tomor 2009
Anser cygnoides (Swan goose)[a]	Minas Gerais, Brazil	*Cryptosporidium baileyi*	50 (1/2)	da Cunha et al. 2017
	Brazil	*Cryptosporidium* spp.	50 (1/2)	Nakamura et al. 2009
Anser fabalis (Bean goose)	Lake Balaton, Hungary	*Cryptosporidium* spp.	12.5 (1/8)	Plutzer and Tomor 2009
Anser sp. (Geese)[a]	Brazil	*Cryptosporidium* spp.	14.3 (3/21)	Nakamura et al. 2009
Aythya fuligula (Tufted duck)	Biesbosch Reservoirs, Netherlands	*Cryptosporidium* spp.	20 (1/5)	Medema 1999
Branta canadensis (Canada goose)	USA	Goose genotype I	17.2 (36/209)	Zhou et al. 2004
	USA	Goose genotype II	4.3 (9/209)	Zhou et al. 2004

Table 1 (continued)

Species name/common name	Geographic origin	Species or genotype	% positive for *Cryptosporidium* spp. (no. positive/no. sampled)	Reference
	USA	Duck genotype	0.5 (1/209)	Zhou et al. 2004
	USA	*Cryptosporidium parvum*	1.9 (4/209)	Zhou et al. 2004
	USA	*Cryptosporidium hominis*	0.9 (2/209)	Zhou et al. 2004
	Ohio, USA	*Cryptosporidium* spp.	81.8 (9/11) / 90 (9/10)	Kassa et al. 2004
	USA	*Cryptosporidium* spp.	6.8 (11/161)	Jellison et al. 2004
Chroicocephalus brunnicephalus (Brown-headed gull)	Bang Poo Nature Reserve, Thailand	Avian genotype III	15.4 (2/13) ***	Koompapong et al. 2014
Chroicocephalus ridibundus (Black-headed gull)	Scotland	*Cryptosporidium* spp.	4.95 (5/101) ****	Smith et al. 1993
	Bang Poo Nature Reserve, Thailand	Avian genotype III	15.4 (2/13) ***	Koompapong et al. 2014
	Biesbosch Reservoirs, Netherlands	*Cryptosporidium* spp.	100 (1/1)	Medema 1999
	Czech Republic	*Cryptosporidium baileyi*	77 (121/157)	Pavlasek 1993
Ciconia ciconia (White stork)	Poland	*Cryptosporidium parvum*	12.5 (3/24)	Majewska et al. 2009
	Galicia, Spain	*Cryptosporidium* spp.	7.7 (1/13)	Reboredo-Fernandez et al. 2015
Cygnus olor (Mute swan)	Poland	*Cryptosporidium parvum*	12.5 (4/33)	Majewska et al. 2009
	Biesbosch Reservoirs, Netherlands	*Cryptosporidium* spp.	25 (1/4)	Medema 1999
Fulica atra (Eurasian Coot)	Lake Balaton, Hungary	*Cryptosporidium parvum*	50 (2/4)	Plutzer and Tomor 2009
Grus vipio (probably *Antigone vipio*) (Whooping crane)[a]	Western Australia	*Cryptosporidium baileyi*	*****	Ng et al. 2006
Larus argentatus (European herring gull)	Scotland	*Cryptosporidium* spp.	4.95 (5/101) ****	Smith et al. 1993
Lophodytes cucullatus (Hooded merganser)	Rio Grande, New Mexico, USA	*Cryptosporidium parvum*	100 (1/1)	Kuhn et al. 2002
Mergus merganser (Common merganser)	Western Poland	*Cryptosporidium parvum*	2.8 (2/72)	Majewska et al. 2009
	Rio Grande, New Mexico, USA	*Cryptosporidium parvum*	66.7 (2/3)	Kuhn et al. 2002
Danish Goose[b]	Germany	*Cryptosporidium* spp.	59.5 (44/74)*	Richter et al. 1994
Phalacrocorax carbo (Great cormorant)	Lake Balaton, Hungary	*Cryptosporidium* spp.	100 (1/1)	Plutzer and Tomor 2009
	Lake Chrzypsko, Poland	*Cryptosporidium* spp.	8 (2/25) ******	Rzymski et al. 2017
	Biesbosch Reservoirs, Netherlands	*Cryptosporidium* spp.	20 (1/5)	Medema 1999
Phoenicopterus ruber (American flamingo)[a]	Western Australia	*Cryptosporidium galli*	*****	Ng et al. 2006
Tadorna ferruginea (Ruddy shelduck)	Qinghai Lake, China	*Cryptosporidium baileyi*	3.38 (5/148)	Amer et al. 2010
Tadorna tadorna (Common shelduck)	Biesbosch Reservoirs, Netherlands	*Cryptosporidium* spp.	25 (2/8)	Medema 1999
Waterbirds	Salburua Wetlands, Spain	Avian genotype III ($n = 4$) Duck genotype b ($n = 1$)	2.3 (6/265) *******	Cano et al. 2016

Table 1 (continued)

Species name/common name	Geographic origin	Species or genotype	% positive for *Cryptosporidium* spp. (no. positive/no. sampled)	Reference
Waterbirds (7 species)	Caspian Sea, Iran	Goose genotype Id ($n = 1$) *Cryptosporidium* spp.	17 (50/293) *	Shemshadi et al. 2014

Species: a = captive birds; b = domestic birds
Faecal samples:
*Samples taken from dead birds
**Total samples (29) = mixed faecal samples *Anser anser domesticus* & *Anas platyrhynchos domesticus*
***Total samples (13) = sets with 70 faecal samples each—mixed faecal samples *Chroicocephalus brunnicephalus* and *C. ridibundus*
****numbers pooled for *Chroicocephalus ridibundus* & *Larus argentatus*
*****Only positive samples ($n = 11$) for *Cryptosporidium* spp. were analysed
******Total samples (25) = pooled faecal samples, estimated from 125 individuals
*******Due to the sampling method, faecal samples could not be assigned to specific bird species

pigeons in Brazil (Oliveira et al. 2017a); 7.03% in turkeys and chickens in Germany (Helmy et al. 2017); 10.20% in farmed chickens in Hubei Province, China (Liao et al. 2018); 12.6% in three chicken production systems in Brazil (Santana et al. 2018); 13.1% in farmed quail in Henan, China (Wang et al. 2012); 14.8% in poultry in Brazil (da Cunha et al. 2018); 15.8% in 3 large farm turkeys flocks in America (McEvoy and Giddings 2009); 25.6% in free-range chickens in Brazil (Ewald et al. 2017); and 50% in domestic ducks in Ahvaz, southwest Iran (Larki et al. 2018). Studies on captive birds have reported prevalence rates of 2.3% in captive birds in Brazil (da Cunha et al. 2017); 3.22% in pet parrots in North China (Zhang et al. 2015); 5% in caged exotic psittacines in Brazil (Ferrari et al. 2018); 9.1% in companion birds in Japan (Iijima et al. 2018); 10.64% in wild captive psittacines in Brazil (Oliveira et al. 2017b); and 19.1% in introduced monk parakeets (*Myiopsitta monachus*) in Santiago, Chile (Briceno et al. 2017). Studies on wild terrestrial avian hosts have reported prevalence rates of 11.7% in wild quail in the rolling plains ecoregion of Texas and Oklahoma, USA (Xiang et al. 2017); 13.42% in Java sparrows (*Lonchura oryzivora*) of northern China (Yao et al. 2017); and 17.1% in North American red-winged blackbirds (*Agelaius phoeniceus*) in the USA (Chelladurai et al. 2016). *Cryptosporidium* seems to be widely dispersed among all avian taxa with implications for transmission of infections to humans via environmental media, such as contamination of water sources by waterbirds, and direct transmission via food and companion birds.

The prevalence of *Cryptosporidium* was between *Cryptosporidium* infection rates found in environmental media, e.g. sewage and river water (2.2%) (Ma et al. 2019) and water samples (27.3%) (Ma et al. 2014b). Compared to studies on livestock, the pattern is varied, with infection rates in livestock ranging from lower values than this study, e.g. yaks (2.53%) (Ren et al. 2019), to mostly similar and some higher rates, e.g. 1–2-month-old highland yaks (11.3%) (Wang et al. 2018a), young domestic farm animals (cattle (14.4%) and sheep (6.2%)) (Zhang et al. 2018b), Tibetan sheep (12.3%) and yaks (28.5%) (Li et al. 2016b), yaks (30.0%) (Ma et al. 2014a), yaks (24.2%) (Mi et al. 2013), farm yaks (12.5%), and farm goats (35.7%) (Karanis et al. 2007) on the QTP in China. Interestingly, similar rates to this study have been found in wild animals, e.g. Qinghai voles (8.9%), and wild plateau pikas (6.25%) (Zhang et al. 2018a), but much higher ones have been found in zoo animals (80%) (Karanis et al. 2007). In comparison, the prevalence of *Giardia* in this study (3.39%) was less than *Giardia* infection rates in environmental media, e.g. water samples (15.4%) (Ma et al. 2014b) and sewage and river waters (21.3%) (Ma et al. 2019) and similar to studies in livestock, e.g. 1–2-month-old highland yaks (5.2%) (Wang et al. 2018a), Zangxiang pigs (6.2%) (Zhang et al. 2019), cattle (10%) (Jian et al. 2018), Tibetan sheep (13.1%) and yaks (10.4%) (Jin et al. 2017), and 4–7-month-

Table 2 Species of waterbirds, origin, and species/genotypes of *Giardia* positives (in %) during the period 1990–today

Species name/common name	Geographic origin	Species or genotype	% positive for *Giardia spp.* (no. positive/no. sampled)	References
Anas acuta (Northern pintail)	Rio Grande, New Mexico, USA	*Giardia* spp.	100 (1/1)	Kuhn et al. 2002
Anas americana (American wigeon)	Rio Grande, New Mexico, USA	*Giardia* spp.	66.7 (2/3)	Kuhn et al. 2002
Anas discors (Blue-winged teal)	Rio Grande, New Mexico, USA	*Giardia* spp.	25 (1/4)	Kuhn et al. 2002
Anas platyrhynchos (Mallard)	Galicia, Spain	*Giardia duodenalis* Assemblage F	50 (2/4)	Reboredo-Fernandez et al. 2015
	Poland	*Giardia lamblia*	21.9 (7/32)	Majewska et al. 2009
	Rio Grande, New Mexico, USA	*Giardia* spp.	25.5 (13/51)	Kuhn et al. 2002
Anas strepera (Gadwall)	Lake Balaton, Hungary	*Giardia duodenalis* Assemblage A	25 (1/4)	Plutzer and Tomor 2009
Anser anser (Greylag goose)	Poland	*Giardia lamblia*	29.4 (10/34)	Majewska et al. 2009
	Lake Balaton, Hungary	*Giardia duodenalis* Assemblage B	2 (1/48)	Plutzer and Tomor 2009
Anser anser domesticus (Greylag goose) [a]	Poland	*Giardia lamblia*	9.1 (1/11)	Majewska et al. 2009
	Hungary	*Giardia* spp.	6.9 (2/29) *	Plutzer and Tomor 2009
Anser fabalis (Bean goose)	Lake Balaton, Hungary	*Giardia* spp.	12.5 (1/8)	Plutzer and Tomor 2009
Ardea herodias (Great blue heron)	USA	*Giardia ardeae*	12.5 (1/8) **	Erlandsen et al. 1990
Balearica pavonina (Black crowned crane) [b]	Poland	*Giardia lamblia*	25 (1/4)	Majewska et al. 2009
Ciconia Ciconia (White stork)	Poland	*Giardia lamblia*	4.2 (1/24)	Majewska et al. 2009
	Netherlands	*Giardia* spp.	100 (1/1)	Franssen et al. 2000
Cygnus olor (Mute swan)	Poland	*Giardia lamblia*	12.5 (4/33)	Majewska et al. 2009
Fulica atra (Eurasian coot)	Lake Balaton, Hungary	*Giardia* spp.	25 (1/4)	Plutzer and Tomor 2009
Mergus merganser (Common merganser)	Poland	*Giardia lamblia*	1.4 (1/72)	Majewska et al. 2009
	Rio Grande, New Mexico, USA	*Giardia* spp.	33.3 (1/3)	Kuhn et al. 2002
Phalacrocorax carbo (Great Cormorant)	Lake Balaton, Hungary	*Giardia* spp.	100 (1/1)	Plutzer and Tomor 2009
Threskiornis spinicollis (Straw-necked ibis)	Australia	*Giardia* spp.	**	Forshaw et al. 1992
	Australia	*Giardia ardeae*	***	McRoberts et al. 1996
Waterbirds	Salburua Wetlands, Spain	*Giardia duodenalis* ($n = 19$) *Giardia duodenalis* sub-assemblage BIV ($n = 2$) *Giardia duodenalis* novel variant ($n = 1$)	8.3 (22/265) ****	Cano et al. 2016
Waterbirds (10 species)	Caspian Sea, Iran	*Giardia lamblia*	24.2 (71/293) **	Shemshadi et al. 2014

Species: a = domestic birds; b = captive birds

Faecal samples:

*Total samples (29) = mixed faecal samples *Anser anser domesticus* and *Anas platyrhynchos domesticus*

**Samples taken from dead birds

***Samples taken from dead birds; *Giardia* spp. was isolated from 63 individuals

****Due to the sampling method, faecal samples could not be assigned to specific bird species

old yaks (5.4%) (Wang et al. 2017) on the QTP in China. The large differences in the prevalence of *Cryptosporidium* spp. and *Giardia* spp. can be attributed to factors including project design and sample collection methods, bird population movement and density, and the intervention of livestock (yaks, cattle, and sheep) and humans.

The prevalence of *Giardia* in this study (3.39%) was generally lower than that reported in previous studies carried out in other locations. In studies on waterbirds (see Table 2), only prevalence rates of 1.4% in common merganser and 4.2% in White stork in Poland (Majewska et al. 2009) and 2% in Greylag geese at Lake Balaton in Hungary (Plutzer and Tomor 2009) were similar to the results of this study. All other studies on waterbirds reported higher prevalence rates (Table 2), ranging from 8.3% in 'waterbirds' (Cano et al. 2016) to 100% in Northern pintail (Kuhn et al. 2002), White stork (Franssen et al. 2000), and great cormorant (Plutzer and Tomor 2009). However, in all cases with 100% infection rates, only one faecal sample was analysed per study. Moreover, infection of wild waterbirds with *Giardia* is widespread but found in less regions than *Cryptosporidium* (Table 2). Studies from Europe include Hungary (Plutzer and Tomor 2009), the Netherlands (Franssen et al. 2000), Poland (Majewska et al. 2009), and Spain (Cano et al. 2016; Reboredo-Fernandez et al. 2015). Studies from other regions are fewer, with two from the USA (Erlandsen et al. 1990; Kuhn et al. 2002) and Australia (Forshaw et al. 1992; McRoberts et al. 1996) and one from Asia (Iran) (Shemshadi et al. 2014).

In other avian taxa, prevalence rates ranged from 1.2% in captive birds in Brazil (da Cunha et al. 2017) and 5.2% in free-ranging, captive, and domestic birds in western Poland (Majewska et al. 2009) to 25.9% in captive Psittaciformes in Brazil (Ichikawa et al. 2019).

From the *Cryptosporidium* species detected in this study, the common species infecting the birds was *C. baileyi* (3 isolates), which was found at two sites and was also identified in yaks from Qinghai Province on the QTP with a prevalence rate of 3.85% (Ren et al. 2019). The zoonotic *C. parvum* genotype (58 isolates) was predominant with 8.54% prevalence; this genotype was also identified in water samples (Ma et al. 2019), yaks (Mi et al. 2013; Wang et al. 2018a), Qinghai voles, wild plateau pikas (Zhang et al. 2018a), and domestic farm animals (cattle and sheep) (Zhang et al. 2018b) on the QTP in China. With respect to *G. duodenalis* detected in this study, 19 isolates were identified as assemblage B, which was also detected in Zangxiang pigs (Zhang et al. 2019) and yaks (Wang et al. 2018a). Importantly, among the eight *G. duodenalis* assemblages, assemblage B is primarily associated with humans, livestock, and wild animals, which suggests that the presence of assemblage B in wild birds is a cause of public health concern. On the other hand, 4 isolates were identified as assemblage E, which was also found in water samples (Ma et al. 2019), Zangxiang pigs (Zhang et al. 2019), yaks (Wang et al. 2018a), cattle (Jian et al. 2018), and Tibetan sheep and yaks (Jin et al. 2017; Wang et al. 2017) on the QTP in China.

Wild birds around Qinghai Lake are mostly migratory; Qinghai Lake is a major breeding site on several migratory bird flyways. Previous studies on the global transmission of avian influenza viruses showed that the virus was spread to Mongolia, Russia, Europe, and Africa along bird migratory flyways (Dong et al. 2017). Similarly, these two zoonotic parasites, *Cryptosporidium* and *Giardia,* may also be transmitted during wild bird migration. Therefore, because of the geographic location of Qinghai Lake and the bird species present in the area, *Cryptosporidium* and *Giardia* may be of public health concern. Importantly, the surrounding areas of Qinghai Lake support human travel and sheep, goats, cattle, and yak grazing, and the water sources are shared with wild animals. In addition, if the wild birds are infected with *Cryptosporidium* and *Giardia*, these parasitic pathogens can spread into or out of the Qinghai Lake area when the wild birds migrate, resulting in a potential threat of further cross-contamination. Therefore, it is imperative to carry out epidemiological investigations in this area.

Conclusions

The 679 faecal samples collected from wild birds in Qinghai Lake areas were screened for the presence of *Cryptosporidium* and *Giardia*. To our knowledge, this is the first study to report a variety of protozoan pathogens (*C. baileyi*, *C. parvum*, and *G. duodenalis* assemblages B and E) in wild birds from the Qinghai Lake area. The results obtained in this study demonstrate the wide prevalence of *Cryptosporidium* and/or *Giardia* in wild birds. Further studies are needed to investigate seasonal effects and the effects of yaks, cattle, sheep, and human environmental factors on the transmission dynamics of *Cryptosporidium* and/or *Giardia* in wild birds on the QTP in China.

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