

# Molecular investigation of *Cryptosporidium* in small caged pets in northeast China: host specificity and zoonotic implications

Qiao Li<sup>1</sup> · Lu Li<sup>2</sup> · Wei Tao<sup>1</sup> · Yanxue Jiang<sup>1</sup> · Qiang Wan<sup>1</sup> · Yongchao Lin<sup>1</sup> · Wei Li<sup>1</sup>

Received: 12 March 2016 / Accepted: 18 April 2016 / Published online: 23 April 2016  
© Springer-Verlag Berlin Heidelberg 2016

**Abstract** This study screened 151 pet-derived fecal specimens randomly collected from four commercial markets in northeast China for the presence of *Cryptosporidium* by genus-specific nested PCRs of the small subunit rRNA gene. Of these, 14 specimens (9.3 %) from nine species of birds, two types of rodents, and a hedgehog were positive for *Cryptosporidium*. Sequence analysis on the PCR-positive isolates facilitated identification of three *Cryptosporidium* species (*C. baileyi*, *C. galli*, and *C. ubiquitum*) and two *Cryptosporidium* genotypes (ferret genotype and avian genotype V). The study birds were affected predominantly with bird-specific *C. baileyi* (Atlantic canary, budgerigar, crested myna, rock dove, and silky fowl), *C. galli* (Chinese hwamei), and *Cryptosporidium* avian genotype V (Fischer's lovebird and rosy-faced lovebird). *Cryptosporidium* ferret genotype previously considered rodent-adapted was identified in three specimens from budgerigar, chipmunk, and red squirrel. Two specimens collected from common hill myna and hedgehog were positive for *C. ubiquitum*. The species of birds that can be colonized by *Cryptosporidium* were extended. Moreover, the data expanded the host range of *Cryptosporidium* ferret genotype and *C. ubiquitum*, especially the birds. The carriage of zoonotic *C. ubiquitum* in small caged pets is of public health importance.

**Keywords** *Cryptosporidium* · Pet animals · Genotyping · Host specificity · Zoonotic potential

## Introduction

Cryptosporidiosis caused by parasites of the genus *Cryptosporidium* (Apicomplexa) is responsible for a significant global burden of diarrhea in humans and animals (Plutzer and Karanis 2009; Xiao 2010). *Cryptosporidium* infections have been reported overwhelmingly in domestic and wild birds and mammals, while our knowledge of the prevalence and genetic characteristics of *Cryptosporidium* in small caged birds and mammals that are commercialized in pet markets is very limited (Feng 2010; Nakamura and Meireles 2015; Plutzer and Karanis 2009; Xiao 2010). Although *Cryptosporidium* is generally recognized as being adapted to specific hosts, co-occurrence of some species (*C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. ubiquitum*, etc.) or genotypes (horse, skunk, monkey genotypes, etc.) in both humans and animals suggests the possibility of zoonotic transmission (Plutzer and Karanis 2009; Slapeta 2013; Xiao and Fayer 2008). A number of different types of small birds and rodents have become increasingly popular as pets worldwide, zoonotic species or genotypes of *Cryptosporidium* carried by them might be an imminent and substantial danger to the public health (Feng 2010; Nakamura and Meireles 2015).

Birds are affected commonly with three *Cryptosporidium* species (*C. baileyi*, *C. galli*, and *C. meleagridis*), five avian genotypes (I to V), five goose genotypes (I to V), the black duck genotype, and the Eurasian woodcock genotype and sporadically with *C. hominis*, *C. parvum*, *C. muris*, etc. (Nakamura and Meireles 2015; Plutzer and Karanis 2009). Among them, *C. meleagridis* is the only zoonotic pathogen and can also infect other mammals (Ryan 2010). Although

✉ Wei Li  
neaulw@gmail.com

<sup>1</sup> College of Veterinary Medicine, Northeast Agricultural University, 59 Mucai street, Harbin, Heilongjiang 150030, China

<sup>2</sup> College of Animal Science and Technology, Henan University of Science and Technology, 70 Tianjin road, Luoyang, Henan 471003, China

one previous report described that an immunodeficient man in Czechoslovakia was colonized with *C. baileyi*, skepticism still remains largely due to the lack of molecular evidence (Ditrich et al. 1991). However, information on host specificity and zoonotic potential of the avian *Cryptosporidium* genotypes is not complete as there exist only a small number of studies involved (Nakamura and Meireles 2015). *Cryptosporidium* infections in wild and domestic rodents have been documented worldwide and these animals are dominantly infected with zoonotic species *C. parvum*, *C. muris*, and *C. ubiquitum* and a set of genotypes (mouse, ferret, mink genotypes, etc.) with strong host specificity (Feng 2010). Among them, *C. ubiquitum* recently renamed from “cervine genotype” is considered a generalist among mammals including humans and has public health significance (Slapeta 2013).

In China, small birds and rodents are popular companions and sold in many pet markets and shops, but molecular survey of *Cryptosporidium* was only performed in central China (Lv et al. 2009; Qi et al. 2011, 2015). Nothing is known about the epidemiology and zoonotic implications of *Cryptosporidium* species or genotypes carried by small caged pets in northeast China. The objective of the current study is to investigate 151 pet animals of various species, breeds, or types for the prevalence and genetic features of *Cryptosporidium* and assess the host specificity and zoonotic potential of the species or genotypes identified.

## Materials and methods

### Specimen collection

Fresh specimens were collected from small caged pets that are commercialized in markets Dafa (eight specimens), Daowai (88 specimens), Hapinglu (43 specimens), and Yongjiu (12 specimens) distributed in districts Xiangfang, Daowai, and Daoli of city Harbin, northeast China (Table 1). The target pets include 27 species of birds (99 specimens), four breeds of rabbits (21 specimens), five types of rodents (26 specimens), turtles (three specimens), and hedgehogs (two specimens) (Table 1). Numbers of specimens in each species, breed, or type of pets were exhibited in Table 1. All the pets sampled were reared in steel cages fitted with trays for excreta collection except that turtles were kept in plastic basins. Only one specimen was collected from each tray or basin whether the pets were kept individually or kept with multiple pets of the same species, breed, or type in a single cage or basin. Specimens were packaged individually in disposable plastic bags with records of the date, location, identification number, clinic presentation, breed, and age. Stools were stored frozen at  $-20^{\circ}\text{C}$  prior to DNA extraction. Pets in Dafa, Daowai, and Yongjiu pet markets were sampled on June 21, 2015, while those from the rest one market on June 22, 2015. All fecal

samples were donated by the pet owners, who granted permission to include those in the survey.

### DNA extraction and nested PCR

Genomic DNA was extracted from 0.2 g (wet weight) pet feces using the StoolGen DNA Kit (Spin-column) (CWBIO, China) and the manufacturer-recommended procedures. PCR amplification was performed using primer sets specific to *Cryptosporidium* that amplified an approximately 830 bp fragment of the small subunit (SSU) rRNA gene as described (Li et al. 2015b). Each specimen was analyzed twice using 2  $\mu\text{l}$  of extracted DNA per PCR. PCRs were performed with a GeneAmp PCR system 9700 thermocycler (Applied Biosystems, Foster City, CA). Aliquots of 5  $\mu\text{l}$  of PCR products were visualized by electrophoresis in 1.5 % agarose with ethidium bromide stain.

### Sequence analysis and species/genotype determination

PCR products showing the expected size were sequenced in both directions at Beijing Genomics Institute, China. Raw sequences were edited to exclude the PCR primer binding sites and manually were corrected using the Chromas Pro version 1.33 (Technelysium Pty. Ltd., Helensvale, Queensland, Australia). For *Cryptosporidium* species/genotype determination, the edited sequences were compared with database sequences by BLAST analysis (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), their similarity was determined based on the degree of sequence identity.

### Statistical analysis

A significant difference in prevalence between animal groups was scored when the  $p$  value was  $<0.05$  using chi-squared test. All tests were analyzed using the SPSS 17.0 (SPSS Inc., Chicago, IL).

## Results

### Prevalence of *Cryptosporidium* in pets

Of the 151 pet specimens, 14 (9.3 %; 95 % confidence interval (CI), 0.044 to 0.141) were positive for *Cryptosporidium* by nested PCRs of the SSU rRNA gene (Table 1). The parasite was found only in two of four pet markets surveyed, with a prevalence of 14.8 % (13/88; 95 % CI, 0.067 to 0.228) in Daowai market and a prevalence of 2.3 % (1/43; 95 % CI,  $-0.022$  to 0.069) in Hapinglu market (Table 1). The difference in prevalence between Daowai and Hapinglu markets is not significant ( $p>0.05$ ,  $\chi^2=3.48$ ). Eleven of 99 bird specimens (11.1 %, 11/99; 95 % CI, 0.045 to 0.177) from nine of 27 bird

**Table 1** Prevalence, species, and genotype of *Cryptosporidium* in small caged pets in northeast China

Class (no.)	Host (no.)	Age (months)	Pet market <sup>a</sup> (no.)	Pathogen (pet market <sup>a</sup> , no.)
Bird (99)	Atlantic canary (3)	10 to 11	DW (3)	<i>C. baileyi</i> (DW, 1)
	Blue swallow (1)	5 to 6	DW (1)	
	Budgerigar (7)	3 to 9	DF (1), DW (3), HP (3)	<i>C. baileyi</i> (DW, 1), ferret genotype (DW, 1)
	Chestnut-flanked white-eye (1)		DW (1)	
	Chinese grosbeak (2)	12 to 36	DW (2)	
	Chinese hwamei (6)		DW (6)	<i>C. galli</i> (DW, 1)
	Common blackbird (1)	7 to 8	DW (1)	
	Common hill myna (29)	2 to 8	DF (3), DW (24), HP (2)	<i>C. ubiquitum</i> (DW, 1)
	Common myna (2)	1 to 3	DW (2)	
	Common nightingale (2)		DW (2)	
	Crested myna (8)	1 to 3	DF (1), DW (2), HP (5)	<i>C. baileyi</i> (DW, 2)
	Eurasian tree sparrow (1)	3 to 4	HP (1)	
	Fischer's lovebird (3)	2 to 3	DW (1), HP (2)	Avian genotype V (HP, 1)
	Hainan blue flycatcher (3)		DW (3)	
	House crow (6)	2 to 3	HP (6)	
	Java sparrow (4)	3	DW (1), HP (3)	
	Long-tailed shrike (1)	18	DW (1)	
	Marsh tit (1)	2 to 3	DW (1)	
	Pied bush chat (1)		DW (1)	
	Rock dove (4)	12	DW (4)	<i>C. baileyi</i> (DW, 1)
	Rosy-faced lovebird (1)		DW (1)	Avian genotype V (DW, 1)
	Siberian rubythroat (4)	3 to 12	DW (1), HP (3)	
	Silky fowl (1)	1 to 2	DW (1)	<i>C. baileyi</i> (DW, 1)
	Sulfur-crested cockatoo (1)	7 to 8	DW (1)	
	Sulfur-breasted warbler (1)	2 to 3	DW (1)	
	Verditer flycatcher (4)	1.5	DW (4)	
	Zebra finch (1)	2 to 3	HP (1)	
Rabbit (21)	Dutch rabbit (2)	8	YJ (2)	
	European rabbit (12)	1 to 2	DW (2), HP (8), YJ (2)	
	Miniature Lop (5)	2 to 3	DF (2), DW (2), YJ (1)	
	Netherland Dwarf (2)	12	HP (2)	
Rodent (26)	Chinchilla (14)	1 to 2	DW (10), YJ (4)	
	Chipmunk (2)	2 to 3	DW (1), YJ (1)	Ferret genotype (DW, 1)
	Guinea pig (4)	1 to 2	HP (4)	
	Hamster (1)		YJ (1)	
	Red squirrel (5)	3 to 4	DW (2), HP (3)	Ferret genotype (DW, 1)
Other (5)	Chinese three-striped box turtle (3)	12 to 96	DF (1), DW (2)	
	Hedgehog (2)		DW (1), YJ (1)	<i>C. ubiquitum</i> (DW, 1)

<sup>a</sup> DF Dafa pet market, DW Daowai pet market, HP Hapinglu pet market, YJ Yongjiu pet market

species (one specimen each for Atlantic canary, Chinese hwamei, common hill myna, Fischer's lovebird, rock dove, rosy-faced lovebird, and silky fowl and two specimens each for budgerigar and crested myna) were detected to be

*Cryptosporidium*-positive (Table 1). The organism was also identified in two of 26 specimens (7.7 %; 95 % CI, −0.030 to 0.148) isolated from two types of rodents (one specimen each for chipmunk and red squirrel) (Table 1). Birds have a

prevalence slightly higher than rodents ( $p > 0.05$ ,  $\chi^2 = 0.02$ ). In addition, one of two specimens from hedgehogs was examined for the presence of *Cryptosporidium* (Table 1). However, none of the *Cryptosporidium* positives was examined in 21 specimens from rabbits and three specimens from turtles (Table 1).

### Species and genotypes of *Cryptosporidium* in pets

Sequencing is available for all 14 *Cryptosporidium*-positive specimens. Among these, only one specimen was from Hapinglu market and the others were from Daowai market (Table 1). Sequence polymorphisms in the SSU rRNA gene and BLAST analysis facilitated identification of five *Cryptosporidium* species and genotypes (Table 1). Of these, *C. baileyi* was seen in one of three specimens from Atlantic canaries aged 10 to 11 months (*Serinus canaria*), one of seven specimens from budgerigars aged 3 to 9 months (*Melopsittacus undulatus*), two of eight specimens from crested mynas aged 1 to 3 months (*Acridotheres cristatellus*), one of four specimens from rock doves aged 12 months (*Columba livia*), and one specimen from silky fowl aged 1 to 2 months (*Gallus gallus domesticus* Brisson) (Table 1). Three *C. baileyi* isolates from budgerigar, crested myna, and rock dove have the DNA sequences identical to a GenBank sequence under accession no. L19068. Compared to L19068 (with the beginning of the first nucleotide as position no. 1), several unreported substitutions were determined in the nucleotide sequences of the rest three *C. baileyi* isolates with G to A change found at position 467 in isolate CHPT14 from crested myna, C to T changes at positions 634, 640, and 696 and A to T change at position 683 in isolate CHPT48 from Atlantic canary, and A to G change at position 675 in isolate CHPT49 from silky fowl. One of three specimens from Fischer's lovebirds aged 2 to 3 months (*Agapornis fischeri*) and one specimen from rosy-faced lovebird (*Agapornis roseicollis*) were identified with *Cryptosporidium* avian genotype V. The two isolates with avian genotype V have 100 % sequence identity to a reported isolate from cockatiel in China (GenBank accession no. HM116381). As seen in Table 1, *C. galli* was found in one of six specimens from Chinese hwamei (*Garrulax canorus*). Compared with a GenBank reference sequence HM116387 (recorded in Silver-eared Liocichla from China), six unreported mutations (G/A, A/G, T/C, A/T, C/T, T/C, and T/G changes at positions 23, 235, 251, 261, 418, 462, and 571, respectively) were observed in the obtained sequence of isolate CHPT18.

Sequence analysis revealed the presence of *C. ubiquitum* in one of 29 specimens from common hill mynas aged 2 to 8 months (*Gracula religiosa*) and one of two specimens from hedgehogs (*Erinaceus europaeus*) (Table 1). *Cryptosporidium* ferret genotype existed in one of seven specimens from budgerigars aged 3 to 9 months (*Melopsittacus undulatus*), one of

two specimens from chipmunks aged 2 to 3 months (*Tamias striatus*), and one of five specimens from red squirrels aged 3 to 4 months (*Sciurus vulgaris*) (Table 1). DNA sequences of the isolates positive for *C. ubiquitum* and ferret genotype are 100 % identical to the existing GenBank reference sequences AF262328 (recorded in water from USA) and AF112572 (recorded in ferret from USA), respectively. Unique nucleotide sequences of the SSU rRNA gene for CHPT14, CHPT48, CHPT49, and CHPT18 were deposited in GenBank under accession numbers KU744845 to KU744848.

### Discussion

To date, quite limited molecular data on *Cryptosporidium* infections in small caged pets have been generated. Molecular investigations of *Cryptosporidium* in pet birds appeared previously in Australia (Ng et al. 2006), Brazil (Nakamura et al. 2009), and central China (Qi et al. 2011). In the former two surveys, sources of the study birds are mixed and the prevalence of *Cryptosporidium* in pet birds is not clear. The current study presented a prevalence of 11.1 % (11/99) in pet birds of 27 species, which is slightly higher than that (8.1 %, 35/434) in pet birds of 31 species in central China. Pet rodents of nine types were previously reported to harbor *Cryptosporidium* at a prevalence rate of 18.1 % (82/452) in central China (Lv et al. 2009; Qi et al. 2015). Our study described the presence of the parasite in two of 26 (7.7 %) pet rodents of five types. There were also several case studies reporting the occurrence of *Cryptosporidium* in pet birds and rodents (Abe and Iseki 2003, 2004; Abe and Makino 2010), which were not included in the prevalence comparison here. *Cryptosporidium* infections occurred in hedgehogs from British (Sangster et al. 2016), Netherlands (Krawczyk et al. 2015), Japan (Abe and Matsubara 2015), Germany (Dyachenko et al. 2010), and Denmark (Enemark et al. 2002), here is the first record of *Cryptosporidium* in Hedgehog in China.

Avian-derived *Cryptosporidium* species can infect a wide range of bird orders, in which *C. baileyi* and *C. meleagridis* are commonly found in the order Galliformes and *C. galli* in the orders Passeriformes and Psittaciformes (Nakamura and Meireles 2015). *C. baileyi* is the most popular species present in the birds of this study that infected five bird species belonging to four orders: Columbiformes (rock dove), Galliformes (silky fowl), Passeriformes (Atlantic canary and crested myna), and Psittaciformes (budgerigar). *C. baileyi* has a strong degree of specificity and was detected in a broad range of bird species worldwide as observed in Table 2. Other than birds, *C. baileyi* was also reported in cattle in Iran and oyster in the USA (Table 2). Since human infection with *C. baileyi* is still doubtful (Ditrich et al. 1991), we could not understand its zoonotic implications. Like *C. baileyi*, as reflected in Table 2, *C. galli* is also very specific to birds and no infection



**Table 2** Host range and geographical distribution of *Cryptosporidium* species/genotypes identified in this study

Pathogen	Host/source: country (host/source type)	Reference
<i>C. baileyi</i>	Human: Czechoslovakia; pet bird: China (black-billed magpie, common myna, crested lark, gold finch, red-billed leiothrix, white java sparrow, zebra finch), Brazil (buffy-fronted seedeater, gold finch, green-winged saltator, lined seedeater, ultramarine grosbeak, white-necked thrush), Czech Republic (crested oropendola, gray-bellied bulbul, red-crowned amazon, red-rumped cacique, rose-ringed parakeet), Japan (cockatiel); other bird: China (chicken, domestic pigeon, ostrich, quail, red-crowned crane, ruddy shelduck), Algeria (chicken), Austria (goose, quail), Brazil (black vulture, chicken, duck, quail, red-cowled cardinal, rufous-bellied thrush, saffron finch), Czech Republic (black-headed gull, channel-billed toucan, chicken, duck, gray partridge, ostrich, red-legged partridge, whooping crane), Germany (mixed-bred falcon, red-breasted merganser), Iran (chicken), Japan (chicken), Korea (chicken), Spain (scops owl), UK (red grouse), USA (chicken, goose, saker falcon); mammal: Iran (cattle); other animal: USA (oyster); water: China (source water, wastewater), Canada (raw water, source water), Germany (source water), Scotland (drinking water, raw water)	(Azami et al. 2007; Ditrich et al. 1991; Fayer et al. 2002; Feng et al. 2011; Hajdusek et al. 2004; Hamidinejat et al. 2014; Kimura et al. 2004; Li et al. 2015a; Nakamura and Meireles 2015; Ruecker et al. 2012; Ryan 2010)
<i>C. galli</i>	Pet bird: China (bohemian waxwing, silver-eared mesia), Australia (canary, chestnut finch, chocolate parson finch, fife canary, gloster, painted firetail finch, turquoise parrot, zebra finch), Brazil (canary, cockatiel, creamy-bellied thrush, double-collared seedeater, goldfinch, great-billed seed-finch, green-winged saltator, hooded siskin, pale-breasted thrush, plain parakeet, ultramarine grosbeak, vinaceous parrot, white-eyed parakeet); other bird: Brazil (chopi blackbird, lesser seed-finch, red-crested cardinal, rufous-bellied thrush, rusty-collared seedeater, rufous-collared sparrow, saffron finch, slate-colored seedeater), Czech Republic (cuban flamingo, hazel hen, red-cowled cardinal, rhinoceros hornbill)	(Nakamura and Meireles 2015)
<i>C. ubiquitum</i>	Human: Canada, England, New Zealand, Nigeria, Peru, Slovenia, Spain, Turkey, UK, USA, Venezuela, Wales; mammal: China (alpine ibex, dog, goat, pet chinchilla, sheep, sika deer, yak), Australia (deer, sheep), Belgium (sheep), Brazil (sheep), Czech Republic (blesbok, mouflon sheep, nyala), France (cattle, goat), Greece (goat, sheep), Italy (cattle), Japan (mouse), Nepal (swamp deer), Norway (sheep), Peru (alpaca), Romania (sheep), Scotland (soay sheep), South Africa (buffalo, impala), Spain (sheep), Sweden (cattle), UK (roe deer, sheep), USA (beaver, cattle, chipmunk, deer mouse, eastern gray squirrel, fox squirrel, gerbil, goat, lemur, mouse, prehensile-tailed opossum, raccoon, red squirrel, sheep, white-tail deer, woodchuck); water: Australia (irrigation catchment, sewage effluent), Canada (source water, raw water), China (raw wastewater), Scotland (drinking water, raw water), Spain (wastewater), UK (environment water), USA (creek, raw wastewater, source water, storm water), Wales (wastewater)	(Abu Samra et al. 2013; Bjorkman et al. 2015; Blanco et al. 2016; Connelly et al. 2013; Fayer et al. 2010; Feng et al. 2012; Galvan et al. 2014; Gomez-Couso et al. 2012; Li et al. 2015b; Molloy et al. 2010; Murakoshi et al. 2013; Nichols et al. 2010; Nolan et al. 2013; Paz e Silva et al. 2014; Qi et al. 2015; Ruecker et al. 2012; Slapeta 2013; Stenger et al. 2015)
Ferret genotype	Mammal: China (siberian chipmunk, red squirrel), Japan (ferret), Italy (red squirrel), USA (black-footed ferret, ferret); water: UK (drinking water)	(Abe and Iseki 2003; Fayer et al. 2010; Lv et al. 2009; Ryan et al. 2014)
Avian genotype V	Pet bird: China (budgerigar, cockatiel), Brazil (blue-fronted parrot), Japan (cockatiel), USA (major mitchell's cockatoo)	(Curtiss et al. 2015; Nakamura and Meireles 2015; Zhang et al. 2015)

reports in other animal species have been documented. It can infect a wide variety of bird species distributed in multiple countries including China (Table 2). This study confirmed the infection of *C. galli* in another passeriform bird, Chinese hwamei. There were only several sporadic reports indicating the affection of *Cryptosporidium* avian genotypes I to V; host specificity and zoonotic potential of the five genotypes are poorly understood (Nakamura and Meireles 2015). As summarized in Table 2, avian genotype V was merely described in several species of psittaciform birds. Likewise, we examined

the genotype in Fischer's lovebird and rosy-faced lovebird belonging to Psittaciformes. Taken together, we can currently conclude that *C. baileyi*, *C. galli*, and avian genotype V are of very limited zoonotic importance. Compared with the published data summarized in Table 2, here represents the first description of *C. baileyi* in Atlantic canary, budgerigar, and silky fowl, *C. galli* in Chinese hwamei, and avian genotype V in Fischer's lovebird and rosy-faced lovebird. Varied levels of unreported nucleotide changes were identified in the SSU rRNA sequences in several isolates of *C. baileyi* and

*C. galli*, which may result from the random amplification of different copies of the SSU rRNA gene in genome or the favorable mutations that allow the pathogens to cope with the variations of bird species and geographic environments.

*Cryptosporidium* ferret genotype, known as a host-specific genotype, normally is restricted to rodent animals like ferret, chipmunk, and red squirrel (Table 2). Identification of the strain in pet rodents in the present study is logical. However, its unwonted presence in a budgerigar indicated a certain degree of host specificity. *C. ubiquitum* has emerged as an important zoonotic pathogen as displayed in Table 2, and a quite wide range of other mammal species are susceptible to infection. The infections of *C. ubiquitum* appear to be spreading both among and across host species and becoming more common than previously thought (Slapeta 2013). But its presence in birds remains unknown. Here is the first report of *C. ubiquitum* in a common hill myna. Hedgehogs are known as a reservoir for zoonotic *C. parvum* and *C. erinacei* (originally recognized as hedgehog genotype) (Abe and Matsubara 2015). The data of this study firstly showed the carriage of *C. ubiquitum* in hedgehog.

In conclusion, this study confirmed the occurrence of zoonotic and potentially host-adapted *Cryptosporidium* species or genotypes in small caged pets from northeast China and revealed some novel polymorphisms in the SSU rRNA gene. The species of birds that can be colonized by *Cryptosporidium* were extended. Moreover, the data expanded the host range of *Cryptosporidium* ferret genotype and *C. ubiquitum*, especially the birds. The carriage of zoonotic *C. ubiquitum* in small caged pets is of public health importance. In addition, as reflected in Table 2, water pollution by *C. baileyi*, ferret genotype, and *C. ubiquitum* should be kept in mind and precautions.

**Acknowledgments** We gratefully acknowledge the financial support from the Young Talents' Project of Northeast Agricultural University (no. 14QC19) and the University Nursing Program for Young Scholars with Creative Talents in Heilongjiang Province (no. UNPYSCT-2015008).

## References

- Abe N, Iseki M (2003) Identification of genotypes of *Cryptosporidium parvum* isolates from ferrets in Japan. *Parasitol Res* 89(5):422–424
- Abe N, Iseki M (2004) Identification of *Cryptosporidium* isolates from cockatiels by direct sequencing of the PCR-amplified small subunit ribosomal RNA gene. *Parasitol Res* 92(6):523–526
- Abe N, Makino I (2010) Multilocus genotypic analysis of *Cryptosporidium* isolates from cockatiels, Japan. *Parasitol Res* 106(6):1491–1497
- Abe N, Matsubara K (2015) Molecular identification of *Cryptosporidium* isolates from exotic pet animals in Japan. *Vet Parasitol* 209(3–4): 254–257
- Abu Samra N, Jori F, Xiao L, Rikhotso O, Thompson PN (2013) Molecular characterization of *Cryptosporidium* species at the wildlife/livestock interface of the Kruger National Park, South Africa. *Comp Immunol Microbiol Infect Dis* 36(3):295–302
- Azami M, Moghaddam DD, Salehi R, Salehi M (2007) The identification of *Cryptosporidium* species (protozoa) in Ifsahan, Iran by PCR-RFLP analysis of the 18S rRNA gene. *Mol Biol (Mosk)* 41(5): 934–939
- Bjorkman C, Lindstrom L, Oweson C, Ahola H, Troell K, Axen C (2015) *Cryptosporidium* infections in suckler herd beef calves. *Parasitology* 142(8):1108–1114
- Blanco MA, de Lucio A, Fuentes I, Carmena D (2016) *Cryptosporidium ubiquitum* in Venezuela: first report in a paediatric patient with acute diarrhoea. *Enferm Infecc Microbiol Clin* 34(2):142–143
- Connelly L, Craig BH, Jones B, Alexander CL (2013) Genetic diversity of *Cryptosporidium* spp. within a remote population of Soay Sheep on St. Kilda Islands, Scotland. *Appl Environ Microbiol* 79(7):2240–2246
- Curtiss JB, Leone AM, Wellehan JF Jr, Emerson JA, Howerth EW, Farina LL (2015) Renal and cloacal cryptosporidiosis (*Cryptosporidium* avian genotype V) in a major mitchell's cockatoo (*Lophochroa leadbeateri*). *J Zoo Wildl Med* 46(4):934–937
- Ditrich O, Palkovic L, Sterba J, Prokopic J, Loudova J, Giboda M (1991) The first finding of *Cryptosporidium baileyi* in man. *Parasitol Res* 77(1):44–47
- Dyachenko V, Kuhnert Y, Schmaeschke R, Etzold M, Pantchev N, Dausgies A (2010) Occurrence and molecular characterization of *Cryptosporidium* spp. genotypes in European hedgehogs (*Erinaceus europaeus* L.) in Germany. *Parasitology* 137(2):205–216
- Enemark HL et al (2002) Molecular characterization of Danish *Cryptosporidium parvum* isolates. *Parasitology* 125(pt 4):331–341
- Fayer R et al (2002) Temporal variability of *Cryptosporidium* in the Chesapeake Bay. *Parasitol Res* 88(11):998–1003
- Fayer R, Santin M, Macarasin D (2010) *Cryptosporidium ubiquitum* n. sp. in animals and humans. *Vet Parasitol* 172(1–2):23–32
- Feng Y (2010) *Cryptosporidium* in wild placental mammals. *Exp Parasitol* 124(1):128–137
- Feng Y et al (2011) Occurrence, source, and human infection potential of *Cryptosporidium* and *Giardia* spp. in source and tap water in shanghai, China. *Appl Environ Microbiol* 77(11):3609–3616
- Feng Y et al (2012) Common occurrence of a unique *Cryptosporidium ryanae* variant in zebu cattle and water buffaloes in the buffer zone of the Chitwan National Park, Nepal. *Vet Parasitol* 185(2–4):309–314
- Galvan AL et al (2014) A year-long study of *Cryptosporidium* species and subtypes in recreational, drinking and wastewater from the central area of Spain. *Sci Total Environ* 468–469:368–375
- Gomez-Couso H et al (2012) Presence and molecular characterisation of *Giardia* and *Cryptosporidium* in alpacas (*Vicugna pacos*) from Peru. *Vet Parasitol* 187(3–4):414–420
- Hajdusek O, Ditrich O, Slapeta J (2004) Molecular identification of *Cryptosporidium* spp. in animal and human hosts from the Czech Republic. *Vet Parasitol* 122(3):183–192
- Hamidinejat H, Jalali MH, Jafari RA, Nourmohammadi K (2014) Molecular determination and genotyping of *Cryptosporidium* spp. in fecal and respiratory samples of industrial poultry in Iran. *Asian Pac J Trop Med* 7(7):517–520
- Kimura A, Suzuki Y, Matsui T (2004) Identification of the *Cryptosporidium* isolate from chickens in Japan by sequence analyses. *J Vet Med Sci* 66(7):879–881
- Krawczyk AI et al (2015) Presence of zoonotic agents in engorged ticks and hedgehog faeces from *Erinaceus europaeus* in (sub) urban areas. *Parasit Vectors* 8:210
- Li J et al (2015a) Molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* in captive wildlife at Zhengzhou zoo, China. *J Eukaryot Microbiol* 62(6):833–839

- Li W et al (2015b) Prevalence and genetic characteristics of *Cryptosporidium*, *Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, China. *Vet Parasitol* 208(3–4):125–134
- Lv C et al (2009) *Cryptosporidium* spp. in wild, laboratory, and pet rodents in China: prevalence and molecular characterization. *Appl Environ Microbiol* 75(24):7692–7699
- Molloy SF et al (2010) Identification of a high diversity of *Cryptosporidium* species genotypes and subtypes in a pediatric population in Nigeria. *Am J Trop Med Hyg* 82(4):608–613
- Murakoshi F et al (2013) Detection and genotyping of *Cryptosporidium* spp. in large Japanese field mice, *Apodemus speciosus*. *Vet Parasitol* 196(1–2):184–188
- Nakamura AA, Meireles MV (2015) *Cryptosporidium* infections in birds—a review. *Rev Bras Parasitol Vet* 24(3):253–267
- Nakamura AA, Simoes DC, Antunes RG, da Silva DC, Meireles MV (2009) Molecular characterization of *Cryptosporidium* spp. from fecal samples of birds kept in captivity in Brazil. *Vet Parasitol* 166(1–2):47–51
- Ng J, Pavlasek I, Ryan U (2006) Identification of novel *Cryptosporidium* genotypes from avian hosts. *Appl Environ Microbiol* 72(12):7548–7553
- Nichols RA, Connelly L, Sullivan CB, Smith HV (2010) Identification of *Cryptosporidium* species and genotypes in Scottish raw and drinking waters during a one-year monitoring period. *Appl Environ Microbiol* 76(17):5977–5986
- Nolan MJ, Jex AR, Koehler AV, Haydon SR, Stevens MA, Gasser RB (2013) Molecular-based investigation of *Cryptosporidium* and *Giardia* from animals in water catchments in southeastern Australia. *Water Res* 47(5):1726–1740
- Paz e Silva FM, Lopes RS, Bresciani KD, Amarante AF, Araujo JP Jr (2014) High occurrence of *Cryptosporidium ubiquitum* and *Giardia duodenalis* genotype E in sheep from Brazil. *Acta Parasitol* 59(1):193–196
- Plutzer J, Karanis P (2009) Genetic polymorphism in *Cryptosporidium* species: an update. *Vet Parasitol* 165(3–4):187–199
- Qi M et al (2011) *Cryptosporidium* spp. in pet birds: genetic diversity and potential public health significance. *Exp Parasitol* 128(4):336–340
- Qi M et al (2015) Zoonotic *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in pet chinchillas (*Chinchilla lanigera*) in China. *Parasitol Int* 64(5):339–341
- Ruecker NJ et al (2012) Molecular and phylogenetic approaches for assessing sources of *Cryptosporidium* contamination in water. *Water Res* 46(16):5135–5150
- Ryan U (2010) *Cryptosporidium* in birds, fish and amphibians. *Exp Parasitol* 124(1):113–120
- Ryan U, Fayer R, Xiao L (2014) *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology* 141(13):1667–1685
- Sangster L et al (2016) Detection and molecular characterisation of *Cryptosporidium parvum* in British European hedgehogs (*Erinaceus europaeus*). *Vet Parasitol* 217:39–44
- Slapeta J (2013) Cryptosporidiosis and *Cryptosporidium* species in animals and humans: a thirty colour rainbow? *Int J Parasitol* 43(12–13):957–970
- Stenger BL et al (2015) North American tree squirrels and ground squirrels with overlapping ranges host different *Cryptosporidium* species and genotypes. *Infect Genet Evol* 36:287–293
- Xiao L (2010) Molecular epidemiology of cryptosporidiosis: an update. *Exp Parasitol* 124(1):80–89
- Xiao L, Fayer R (2008) Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. *Int J Parasitol* 38(11):1239–1255
- Zhang XX, Zhang NZ, Zhao GH, Zhao Q, Zhu XQ (2015) Prevalence and genotyping of *Cryptosporidium* infection in pet parrots in north China. *Biomed Res Int* 2015:549798