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ORIGINAL PAPER

Molecular identification of *Enterocytozoon bieneusi*, *Cryptosporidium*, and *Giardia* in Brazilian captive birds

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Abstract A total of 85 fecal samples from captive birds collected from October 2013 to September 2014 in Uberlândia and Belo Horizonte in the state of Minas Gerais (Brazil) were evaluated for the presence of *Enterocytozoon bieneusi*, *Cryptosporidium*, and *Giardia* by PCR. Of these, three birds were found positive for *E. bieneusi* (3.5%), two for *Cryptosporidium* (2.3%), and one for *Giardia* (1.2%). Two genotypes of *E. bieneusi* were detected by nucleotide sequence analysis of the ITS region, genotypes D and Peru 6 in a swan goose and in two rock pigeons, respectively. For *Cryptosporidium* and *Giardia*, nucleotide sequence analysis of the SSU rRNA identified *Cryptosporidium baileyi* and Duck genotype in a swan goose and a mandarin duck, respectively, and *Giardia duodenalis* assemblage A in a toco toucon. Our results demonstrate that human-pathogenic *E. bieneusi* genotypes D and Peru6 and *G. duodenalis* assemblage A are present in captive birds in Brazil, corroborating their potential role as a source of human infection and environmental contamination.

Keywords Birds · *Cryptosporidium* · *Enterocytozoon bieneusi* · Genotypes · *Giardia* · Zoonotic

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Introduction

Enterocytozoon bieneusi, *Cryptosporidium*, and *Giardia* are environmentally ubiquitous enteropathogens that cause serious human and animal diarrheal diseases (Ryan and Caccio 2013; Ryan et al. 2014; Santín 2015).

Cryptosporidiosis is one of the most prevalent parasitic infections in domesticated, caged, and wild birds worldwide (O'Donoghue 1995; Sréter and Varga 2000; Wang et al. 2014). More than 30 avian species has been reported infected with *Cryptosporidium* (Ryan 2010). So far, four *Cryptosporidium* species have been identified in birds, *C. baileyi*, *C. meleagridis*, *C. galli*, and *C. avium* (Slavin 1955; Current et al. 1986; Ryan et al. 2003; Holubová et al. 2016). Each of them can infect many avian species, but they differ in their host range, infection sites, and symptomatology associated with infection. In addition to these four species, 13 *Cryptosporidium* genotypes have been described in birds worldwide, including avian genotypes I–VI, goose genotypes I–V, black duck genotype, and Eurasian woodcock genotype (Nakamura and Meireles 2015; Chelladurai et al. 2016). Among them, only *C. meleagridis* is known to also infect humans and has public health significance (Xiao 2010).

Giardia have been reported in wild and captive birds worldwide (Erlandsen et al. 1991; McRoberts et al. 1996; Abe et al. 2012; Papini et al. 2012; Reboredo-Fernández et al. 2015). Two species of *Giardia* are responsible of avian giardiasis, *G. ardeae* and *G. psittaci* (Ryan and Caccio 2013). In addition to those two avian species, *Giardia duodenalis* has also been reported in birds. Thus far, zoonotic assemblages A and B as well as nonzoonotic assemblages D and F have been found in birds (Reboredo-Fernández et al. 2015; Majewska et al. 2009).

Enterocytozoon bieuneusi is an emerging opportunistic human pathogen included in the phylum Microsporidia. Besides humans, *E. bieneusi* has been found in a broad range

of domestic and wild animals (Santín and Fayer 2011). Genetic studies have demonstrated the presence of more than 200 genotypes, some have been found only in humans or only in animal hosts, but several have been found in both humans and animals, suggestion zoonotic potential (Santín 2015). Multiple *E. bieneusi* genotypes, including those with zoonotic potential, have been identified in birds worldwide (Müller et al. 2008; Kasicková et al. 2009; Galvan-Díaz et al. 2014; da Cunha et al. 2016; Zhao et al. 2016).

Although it has been indicated that captive birds could play an important role in the transmission of zoonotic parasites *Cryptosporidium*, *E. bieneusi*, and *Giardia* for humans and other animals (Kasicková et al. 2009; Majewska et al. 2009; Papini et al. 2012; Pirestani et al. 2013), there is limited information in the literature on the presence of these parasites in captive birds worldwide. The present study was designed to determine the presence of species and genotypes of *E. bieneusi*, *Cryptosporidium*, and *Giardia* in captive birds from the state of Minas Gerais (Brazil).

Material and methods

Samples

From October 2013 to September 2014, a total of 85 fecal specimens were collected from captive birds at the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) ($n = 15$), a private aviary ($n = 6$), a school of falconry ($n = 10$), and nine local markets ($n = 54$) in Uberlândia and Belo Horizonte in the state of Minas Gerais, Brazil. The birds included 9 orders and 33 species (Table 1). At the time of sampling, all birds examined were apparently in good health and no diarrhea was observed. To collect the samples, the animals were placed in individual sanitized cages and fresh feces were collected from the bottom of the cages. Fecal specimens were placed into sterile polystyrene tubes with records of the date, location, identification number, and species, and transferred in isothermal boxes to the Parasitology Laboratory of Federal University of Uberlândia (UFU) and held at −20 °C until DNA extraction.

DNA extraction, PCR, and sequencing

Genomic DNA was extracted directly from each individual fecal specimen using the QIAamp Stool Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instruction with minor modifications. Modifications included the addition of 0.3 g of zirconia beads (Stratech Scientific, Luton, UK) to 0.2 g of feces and 1.4 ml lysis buffer (McLauchlin et al. 1999); then, the mixture was heated at 95 °C for 5 min followed by vigorous shaking (two rounds of 15 min) to facilitate the parasite rupture. The nucleic acid was eluted in 150 μl of AE buffer to increase the quantity of DNA recovered.

Polymerase chain reaction protocols for amplifying gene fragments for *Cryptosporidium* (SSU rRNA), *Giardia* (SSU rRNA), and *E. bieneusi* (SSU rRNA, ITS, and LSU rRNA) have been previously described (Hopkins et al. 1997; Xiao et al. 1999; Buckholt et al. 2002). Negative and positive controls were included in all PCR sets. Secondary PCR products were detected and visualized by electrophoresis in the QIAxcel Advanced System (Qiagen, Valencia, CA). All positive PCR products were purified using Exonuclease I/Shrimp Alkaline Phosphatase (Exo-SAP-ITTM) (USB Corporation, Cleveland, OH) and sequenced in both directions using the same PCR primers used in the secondary PCR in 10 μl reactions using Big Dye™ chemistries and an ABI 3130 sequencer analyzer (Applied Biosystems, Foster City, CA). Nucleotide sequences obtained in this study were aligned, examined, and compared with reference sequences from GenBank using SeqMan™ (DNAstar Inc., Madison, WI). The nucleotide sequences obtained in this study have been deposited in GenBank under accession numbers KY012352–KY012356.

Results

Of the 85 bird specimens, 3 (3.5%), 2 (2.3%), and 1 (1.2%) were PCR-positive for *E. bieneusi*, *Cryptosporidium*, and *G. duodenalis*, respectively (Table 1). For E. *bieneusi*, the three positive birds were a swan goose from market 3 in Uberlândia and two rock pigeons from market 7 in Belo Horizonte. *Crypstoporidium* was found in a swan goose and a mandarin duck from markets 2 and 3 in Uberlândia, respectively. *Giardia* was identified in a toco toucon from a private aviary in Uberlândia. All birds examined from the School of Falconry, IBAMA, and markets 1, 4, 5, 6, 8, and 9 were negative for the three parasites. *E. bieneusi* was found in birds from Uberlândia and Belo Horizonte while *Cryptosporidium* and *Giardia* were only identified in birds from Uberlândia.

All PCR positive samples were successfully sequenced, and no mixed infections were observed (Table 1). For *E. bieneusi*, nucleotide sequence analysis of the ITS revealed two distinct genotypes, genotype D in a swan goose and Peru 6 in two rock pigeons. For *Cryptosporidium*, *C. baileyi* was found in a swan goose and Duck genotype in a mandarin duck sample. For *G. duodenalis*, assemblage A was identified in a toco toucon.

Discussion

This study demonstrated the presence of *E. bieneusi*, *Cryptosporidium*, and *G. duodenalis* in captive birds in Brazil. This is the first report of *E. bieneusi* in captive birds

Table 1 Prevalence and genotyping results for *Cryptosporidium*, *Giardia*, and *Enterocytozoon bieneusi* in captive birds in Uberlândia and Belo Horizonte in the state of Minas Gerais (Brazil)

Location		Order	Scientific name (common name)	No. examined	No. of *E. bieneusi* positives (genotype)	No. of *Cryptosporidium* positives (Species/genotype)	No. of *G. duodenalis* positives (assemblage)
Uberlândia	School of Falconry	Falconiformes	*Falco femoralis* (aplomado falcon)	6	0	0	0
		Strigiformes	*Bubo virginianus* (great horned owl)	1	0	0	0
			Tyto alba (American barn owl)	2	0	0	0
		Accipitriformes	*Buteo albicaudatus* (white-tailed hawk)	1	0	0	0
	Aviary	Psittaciformes	*Agapornis nigrigenis* (black-cheeked lovebird)	1	0	0	0
			Agapornis roseicollis (peach-faced lovebird)	1	0	0	0
			Nymphicus hollandicus (cockatie)	3	0	0	0
		Piciformes	*Ramphastos toco* (toco toucan)	1	0	0	1 (assemblage A)
	Market 1	Anseriformes	*Cairina moschata* (Muscovy duck)	2	0	0	0
	Market 2	Anseriformes	*Anser cygnoides* (swan goose)	2	0	1 (*C. baileyi*)	0
			Anas querquedula (garganey)	1	0	0	0
		Galliformes	*Pavo cristatus* (Indian peafowl)	1	0	0	0
		Psittaciformes	*Aratinga leucophthalma* (white-eyed parakeet)	1	0	0	0
			Melopsittacus undulatus (budgerigar)	1	0	0	0
	Market 3	Anseriformes	*Anser cygnoides* (swan goose)	1	1 (D)	0	0
			Aix galericulata (mandarin duck)	1	0	1 (Duck genotype)	0
			Anas platyrhynchos (wild duck)	1	0	0	0
			Cairina moschata (Muscovy duck)	1	0	0	0
	Market 4	Anseriformes	*Cairina moschata* (Muscovy duck)	1	0	0	0
		Passeriformes	*Serinus canaria* (wild canary)	8	0	0	0
	Market 5	Psittaciformes	*Melopsittacus undulatus* (budgerigar)	1	0	0	0
	Market 6	Anseriformes	*Callonetta leucophrys* (ringed teal)	1	0	0	0
			Aix galericulata (mandarin duck)	1	0	0	0
Belo Horizonte	IBAMA	Psittaciformes	*Amazona aestiva* (blue-fronted parrot)	3	0	0	0
			Aratinga leucophthalma (white-eyed parakeet)	1	0	0	0
		Passeriformes	*Saltator similis* (green-winged saltator)	9	0	0	0
			Saltatricula atricollis (black-throated saltator)	1	0	0	0
			Schistochlamys ruficapillus (cinnamon tanager)	1	0	0	0
	Market 7	Anseriformes	*Cairina moschata* (Muscovy duck)	2	0	0	0
			Anas querquedula (garganey)	1	0	0	0
		Columbiformes	*Columba livia* (rock pigeon)	2	2 (Peru6)	0	0

Table 1 (continued)

Location		Order	Scientific name (common name)	No. examined	No. of *E. bieneusi* positives (genotype)	No. of *Cryptosporidium* positives (Species/genotype)	No. of *G. duodenalis* positives (assemblage)
	Market 8	Galliformes	*Pavo cristatus* (Indian peafowl)	2	0	0	0
		Psittaciformes	*Pyrrhura perlata* (crimson-bellied parakeet)	3	0	0	0
			Pyrrhura roseifrons (rose-fronted parakeet)	1	0	0	0
			Psittacula krameri (ringneck parakeet)	4	0	0	0
			Pionus menstruus (blue-headed parrot)	1	0	0	0
			Aratinga jandaya (Jandaya parakeet)	2	0	0	0
			Ara chloropterus (red-and-green macaw)	1	0	0	0
			Amazona aestiva (blue-fronted parrot)	2	0	0	0
			Pyrrhura cruentata (blue-throated parakeet)	1	0	0	0
	Market 9	Passeriformes	*Serinus canaria* (wild canary)	2	0	0	0
			Sicalis flaveola (saffron finch)	2	0	0	0
			Sporophila caerulescens (double-collared seedeater)	2	0	0	0
Total				85	3	2	1

in Brazil where, so far, it only been reported in chickens, free-living pigeons, and exotics birds (Lallo et al. 2012; da Cunha et al. 2016). Of the three parasites studied, *E. bieneusi* was the most frequent with a prevalence of 3.2%. The prevalence was similar to the one previously reported in free-living pigeons and exotic birds in Brazil (5.6%), but lower than the one reported in chickens in Brazil (15.9%) and those reported in various birds in studies in Portugal (29%), China (22.2%), Czech Republic (12.5%), and Spain (9.7%) (Haro et al. 2006; Lobo et al. 2006; Kasicková et al. 2009; Lallo et al. 2012; da Cunha et al. 2016; Zhao et al. 2016). In this study, *E. bieneusi* was identified in a swan goose and in two pigeons expanding the host range of this parasite in Brazil as it is the first time this parasite was found in birds of the order Anseriformes in this country. In the order Anseriformes, there is only another report of *E. bieneusi* in farmed domestic geese (*Anser domestica*) and domestic duck (*Anas platyrhynchos domesticus*) in China (Zhao et al. 2016). However, *E. bieneusi* has been commonly detected in pigeons with reports in Brazil, China, Iran, Netherlands, Spain, Poland, and Portugal (Haro et al. 2006; Lobo et al. 2006; Bart et al. 2008; Lallo et al. 2012; Pirestani et al. 2013; Stodkowicz-Kowalska et al. 2013; Zhao et al. 2016).

The nucleotide sequence analysis of the ITS region of the *E. bieneusi* isolates revealed the presence of two genotypes, D in the swan goose and Peru 6 in the two rock pigeons. Both genotypes belong to group 1 which predominately infects humans and has zoonotic importance (Santín and Fayer 2011). Genotypes D and Peru 6 have been widely reported in humans as well as in a wide range of animal hosts worldwide (Santín 2015). In Brazil, genotype D was found in humans and cattle (Feng et al. 2011; da Silva Fiuza et al. 2016) and Peru 6 in chickens (da Cunha et al. 2016). Genotype D has previously identified in avian hosts of the orders Falconiformes in Abu Dhabi (Müller et al. 2008), Gruiformes in China (Zhao et al. 2016), Columbiformes in Iran (Pirestani et al. 2013), and Galliformes in Brazil (da Cunha et al. 2016). Similarly, Peru 6 has been previously found in orders Psittaciformes and Columbiformes in Portugal (Lobo et al. 2006), Galliformes in Brazil (da Cunha et al. 2016), and Gruiformes in China (Zhao et al. 2016).

Cryptosporidium has been reported in a wide range of domestic, wild and captive avian hosts in worldwide (Ryan 2010; Nakamura and Meireles 2015). In Brazil, it has been previously reported in domestic, wild, exotic, and captive birds (Huber et al. 2007; Nakamura et al. 2009, 2014; Sevá et al. 2011; Gomes et al. 2012; Holsback et al. 2013; da Cunha et al. 2016). In this study, *Cryptosporidium* was detected in 2 (2.1%) of the 85 fecal samples examined. A similar prevalence was reported in domestic pigeons in Iran (2.94%). However, it was higher than the prevalence found in pigeons from China (0.8%) and a lower than the one reported in domestic and captive birds in studies in Brazil and other countries (Papini et al. 2012; Nakamura et al. 2014; Nakamura and Meireles 2015; Li et al. 2016). Molecular characterization identified *C. baileyi* in a swan goose and the Duck genotype in a mandarin duck. *C. baileyi* is considered the most common avian *Cryptosporidium* species worldwide (Nakamura et al. 2009; Ryan 2010). In Brazil, it has been previously reported in wild, captive, and domestic birds of the orders Passeriformes, Anseriformes, Galliformes, and Cathartiformes (Huber et al. 2007; Nakamura et al. 2009, 2014; Sevá et al. 2011). However, this is the first time that the Duck genotype has been reported in South America, until now, it was only reported twice in North America in Canada geese (Jellison et al. 2004; Zhou et al. 2004).

Although *Giardia* has been widely documented in mammals, there is limited number of studies in birds with no reports of *Giardia* in birds in Brazil. The presence of *Giardia* in a toco toucan constitutes not only the first report of *Giardia* in birds in Brazil but also the first report of *Giardia* in a bird from the order Piciformes. This parasite has been identified in various orders of captive and wild birds (Papini et al. 2012; Reboredo-Fernández et al. 2015; Cano et al. 2016). The overall *Giardia* prevalence was low (1.1%) and similar to prevalences reported in pet birds (1.6%) and zoo birds (3.6%) in Italy (Papini et al. 2012), and wild birds in Spain (2.1%) (Reboredo-Fernández et al. 2015). Molecular characterization of the toco toucan isolate identified *G. duodenalis* assemblage A. This assemblage is known to be infectious for humans and for a wide range of mammals. In birds, assemblage A has previously been reported in wild birds in Spain (Reboredo-Fernández et al. 2015), domestic birds in Ivory Coast (Berrilli et al. 2012), and captive birds in Italy (Papini et al. 2012). *G. duodenalis* assemblages B, D, and F have also been identified in wild and aquatic birds in Spain (Reboredo-Fernández et al. 2015; Cano et al. 2016) and domestic birds in Ivory Coast (Berrilli et al. 2012).

In conclusion, this study confirmed the occurrence of zoonotic parasites in captive birds in Brazil raising questions on potential zoonotic transmission of *G. duodenalis* and *E. bieneusi* from captive birds to humans. All positive birds were apparently healthy and might serve as asymptomatic carriers of these parasites. Birds keep in captivity are in close contact with humans and have to be periodically handled for feeding and sanitation, and all those activities could expose handlers to infection by these and others zoonotic parasites. In addition, if waste is not properly handled, it could contaminate water and food and contribute to the indirect transmission of these parasites.

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References

Abe N, Makino I, Kojima A (2012) Molecular characterization of *Giardia psittaci* by multilocus sequence analysis. Infect Genet Evol 12:1710–1716

Bart A, Wentink-Bonnema EM, Heddema ER, Buijis J, van Gool T (2008) Frequent occurrence of humam-associated microsporidia in fecal droppings of urban pigeons in Amsterdam, the Netherlands. Appl Environ Microbiol 74:7056–7058

Berrilli F, D'Alfonso R, Giangaspero A, Marangi M, Brandonisio O, Kaboré Y, Glé C, Cianfanelli C, Lauro R, Di Cave D (2012) *Giardia duodenalis* genotypes and *Cryptosporidium* species in humans and domestic animals in Côte d'Ivoire: occurrence and evidence for environmental contamination. Trans R Soc Trop Med Hyg 106:191–195

Buckholt MA, Lee JH, Tzipori S (2002) Prevalence of *Enterocytozoon bieneusi* in swine: an 18-month survey at a slaughterhouse in Massachusetts. Appl Environ Microbiol 68:2595–2599

Cano L, de Lucio A, Bailo B, Cardona GA, Muadica ASO, Lobo L, Carmena D (2016) Identification and genotyping of *Giardia* spp. and *Cryptosporidium* spp. isolates in aquatic birds in the Salburua wetlands, Álava, Northern Spain. Vet Parasitol 221:144–148

Chelladurai JJ, Clark ME, Kváč M, Holubová N, Khan E, Stenger BL, Giddings CW, McEvoy J (2016) *Cryptosporidium galli* and novel *Cryptosporidium* avian genotype VI in North American red-winged blackbirds (*Agelaius phoeniceus*). Parasitol Res 115:1901–1906

Current WL, Upton SJ, Haynes TB (1986) The life cycle of *Cryptosporidium baileyi* n. sp. (Apicomplexa, Cryptosporidiidae) infecting chickens. J Protozool 33:289–296

da Cunha MJ, Cury MC, Santín M (2016) Widespread presence of human-pathogenic *Enterocytozoon bieneusi* genotypes in chickens. Vet Parasitol 217:108–112

da Silva Fiuza VR, Lopes CW, de Oliveira FC, Fayer R, Santín M (2016) New findings of *Enterocytozoon bieneusi* in beef and dairy cattle in Brazil. Vet Parasitol 216:46–51

Erlandsen SL, Weisbrod AR, Knudson LW, Olereich R, Dodge WE, Jakubowski W, Bemrick WJ (1991) Giardiasis in wild and captive bird populations: High prevalence in herons and budgerigars. Int J Environ Health Res 1:132–143

Feng Y, Li N, Dearen T, Lobo ML, Matos O, Cama V, Xiao L (2011) Development of a multilocus sequence typing tool for high-resolution genotyping of *Enterocytozoon bieneusi*. Appl Environ Microbiol 77:4822–4828

Galvan-Díaz AL, Magnet A, Fenoy S, Henriques-Gil N, Haro M, Gordo FP, Millán J, Miró J, del Águila C, Izquierdo F (2014) Microsporidia detection and genotyping study of human pathogenic *E. bieneusi* in animals from Spain. Plos One 9:e92289

Gomes RS, Huber F, da Silva S, do Bomfim TCB (2012) *Cryptosporidium* spp. parasitize exotic birds that are commercialized in markets, commercial aviaries, and pet shops. Parasitol Res 110:1363–1370

Haro M, Henriques-Gil N, Fenoy S, Izquierdo F, Alonso F, Del Aguila C (2006) Detection and genotyping of *Enterocytozoon bieneusi* in pigeons. J Eukaryot Microbiol 53:S58–S60

Holsback L, Cardoso MJ, Fagnani R, Patelli TH (2013) Natural infection by endoparasites among free-living wild animals. Rev Bras Parasitol Vet 22:302–306

Holubová N, Sak B, Horčičková M, Hlásková L, Květoňová D, Menchaca S, McEvoy J, Kváč M (2016) *Cryptosporidium avium* n. sp. (Apicomplexa: Cryptosporidiidae) in birds. Parasitol Res 115: 2243–2251

Hopkins RM, Meloni BP, Groth DM, Wetherall JD, Reynoldson JA, Thompson RCA (1997) Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. J Parasitol 83:44–51

Huber F, da Silva S, Bonfim TCB, Teixeira KRS, Bello AR (2007) Genotypic characterization and phylogenetic analysis of *Cryptosporidium* sp. from domestic animals in Brazil. Vet Parasitol 150:65–74

Jellison KL, Distel DL, Hemond HF, Schauer DB (2004) Phylogenetic analysis of the hypervariable region of the 18S rRNA gene of *Cryptosporidium* oocysts in feces of Canada geese (*Branta canadensis*): evidence for five novel genotypes. Appl Environ Microbiol 70:452–458

Kasicková D, Sak B, Kváč M, Ditrich O (2009) Sources of potentially infectious human microsporidia: molecular characterization of microsporidia isolates from exotic birds in the Czech Republic, prevalence study and importance of birds in epidemiology of the human microsporidial infections. Vet Parasitol 165:125–130

Lallo MA, Calábria P, Milanelo L (2012) *Encephalitozoon* and *Enterocytozoon* (Microsporidia) spores in stool from pigeons and exotic birds. Vet Parasitol 190:418–422

Li Q, Li L, Tao W, Jiang Y, Wan Q, Lin Y, Li W (2016) Molecular investigation of *Cryptosporidium* in small caged pets in northeast China: host specificity and zoonotic implications. Parasitol Res 15:2905–2911

Lobo ML, Xiao L, Cama V, Magalhaes N, Antunes F, Matos O (2006) Identification of potentially human-pathogenic *Enterocytozoon bieneusi* genotypes in various birds. Appl Environ Microbiol 72:7380–7382

Majewska AC, Taddeus KG, Słodkowicz-Kowalska A, Tamang L, Jędrzejewski S, Zduniak P, Solarczyk P, Nowosad A, Nowosad P (2009) The role of free-ranging, captive, and domestic birds of Western Poland in environmental contamination with *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts. Parasitol Res 104:1093–1099

McLauchlin J, Pedraza-Díaz S, Amar-Hoetzeneder C, Nichols GL (1999) Genetic characterization of *Cryptosporidium* strains from 218 patients with diarrhea diagnosed as having sporadic cryptosporidiosis. J Clin Microbiol 37:3153–3158

McRoberts KM, Meloni BP, Morgan UM, Marano R, Binz N, Eriandsen SL, Halse SA, Thompson RC (1996) Morphological and molecular characterization of *Giardia* isolated from the straw-necked ibis (*Threskiornis spinicollis*) in Western Australia. J Parasitol 82:711–718

Müller MG, Kinne J, Schuster RK, Walochnik J (2008) Outbreak of microsporidiosis caused by *Enterocytozoon bieneusi* in falcons. Vet Parasitol 152:67–78

Nakamura AA, Meireles MV (2015) *Cryptosporidium* in birds - a review. Braz J Vet Parasitol 24:253–267

Nakamura AA, Simões 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 166:47–51

Nakamura AA, Homem CG, da Silva AMJ, Meireles MV (2014) Diagnosis of gastric cryptosporidiosis in birds using a duplexreal-time PCR assay. Vet Parasitol 205:7–13

O'Donoghue PJ (1995) *Cryptosporidium* and cryptosporidiosis in man and animals. Int J Parasitol 25:139–195

Papini R, Girivetto M, Marangi M, Mancianti F, Giangaspero A (2012) Endoparasite infection in pet and zoo Birds in Italy. Sci World J 2012:1–9

Pirestani M, Sadraei J, Forouzandeh M (2013) Molecular characterization and genotyping of human related microsporidia in free-ranging and captive pigeons of Tehran, Iran. Infect Genet Evol 20:495–499

Reboredo-Fernández A, Ares-Mazás E, Caccio SM, Gómez-Couso H (2015) Occurrence of *Giardia* and *Cryptosporidium* in wild birds in Galicia (Northwest Spain). Parasitology 142:917–925

Ryan U (2010) *Cryptosporidium* in birds, fish and amphibians. Exp Parasitol 124:113–120

Ryan U, Caccio SM (2013) Zoonotic potential of *Giardia*. Int J Parasitol 43:943–956

Ryan UM, Xiao L, Read C, Sulaiman IM, Monis P, Lal AA, Fayer R, Pavlásek I (2003) A redescription of *Cryptosporidium galli* Pavlasek, 1999 (Apicomplexa: Cryptosporidiidae) from birds. J Parasitol 89:809–813

Ryan U, Fayer R, Xiao L (2014) *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology 141:1667–1685

Santín M (2015) Enterocytozoon bieneusi. In: Xiao L, Ryan U (eds) Biology of Foodborne Parasites, 1st edn. CRC Press, Boca Raton, pp 149–174

Santín M, Fayer R (2011) Microsporidiosis: Enterocytozoon bieneusi in domesticated and wild animals. Res Vet Sci 90:363–371

Sevá AP, Funada MR, Richtzenhain L, Guimarães MB, Souza SO, Allegretti L, Sinhorini JA, Duarte VV, Soares M (2011) Genotyping of *Cryptosporidium* spp. from free-living wild birds from Brazil. Vet Parasitol 175:27–32

Slavin D (1955) *Cryptosporidium meleagridis* (sp. nov.). J Comp Pathol 65:262–266

Sréter T, Varga I (2000) Cryptosporidiosis in birds—a review. Vet Parasitol 87:261–279

Stodkowicz-Kowalska A, Graczyk TK, Nowosad A, Majewska AC (2013) First detection of microsporidia in raised pigeons in Poland. Ann Agric Environ Med 20:13–15

Wang L, Xue X, Li J, Zhou Q, Yu Y, Du A (2014) Cryptosporidiosis in broiler chickens in Zhejiang Province, China: molecular characterization of oocysts detected in fecal samples. Parasite 21:36

Xiao L (2010) Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol 124:80–88

Xiao L, Morgan U, Limor J, Escalante A, Arrowood M, Shulaw W, Thompson RC, Fayer R, Lal AA (1999) Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl Environ Microbiol 65:3386–33918

Zhao W, Yu S, Yang Z, Zhang Y, Zhang L, Wang R, Zhang W, Yang F, Liu F (2016) Genotyping of *Enterocytozoon bieneusi* (Microsporidia) isolated from various birds in China. Infec Genet Evol 40:151–154

Zhou L, Kassa H, Tischler ML, Xiao L (2004) Host-adapted *Cryptosporidium* spp. in Canada geese (*Branta canadensis*). Appl Environ Microbiol 70:4211–4215