Occurrence of *Giardia* and *Cryptosporidium* in wild birds in Galicia (Northwest Spain)

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SUMMARY

Faecal samples were obtained from 433 wild birds being treated in wildlife recovery centres in Galicia (Northwest Spain), between February 2007 and September 2009. The birds belonged to 64 species representing 17 different orders. *Giardia* cysts and *Cryptosporidium* oocysts were detected by an immunofluorescence antibody test and identified at the molecular level by established PCR-sequencing methods. The overall prevalence of *Giardia* was 2·1% and that of *Cryptosporidium*, 8·3%. To our knowledge, this is the first description of *Giardia* sp. in *Tyto alba* and *Caprimulgus europaeus*; and of *Cryptosporidium* sp. in *Apus apus, Athene noctua, C. europaeus, Falco tinnunculus, Morus bassanus, Parabuteo unicinctus* and *Strix aluco*. Furthermore, the first PCR-sequence confirmed detection of *Giardia duodenalis* assemblage B in, *Buteo buteo, Coturnix coturnix* and *Pica pica; G. duodenalis* assemblage D in *Garrulus glandarius;* and *G. duodenalis* assemblage F in *Anas platyrhynchos; Cryptosporidium parvum* in *Accipiter nisus, B. buteo, Milvus migrans, Pernis apivorus* and *P. pica;* and *Cryptosporidium meleagridis* in *Streptopelia turtur*. The study findings demonstrate the wide spread of *Giardia* and *Cryptosporidium* between wild birds.

Key words: wild birds, *Giardia*, *Cryptosporidium*, immunofluorescence microscopy, molecular characterization, Galicia (Northwest Spain).

INTRODUCTION

Giardia spp. and *Cryptosporidium* spp. are protozoan parasites that infect a wide range of vertebrate hosts, including humans, domestic and wild animals (Xiao, 2010; Feng and Xiao, 2011). Currently, 2 of the 6 species of *Giardia* are recognized in avian hosts on the basis of the morphology of trophozoites and/or cysts: *Giardia ardeae* and *Giardia psittaci* (Ryan and Cacciò, 2013). Likewise, 3 species of *Cryptosporidium* have been reported in birds on the basis of biological and genetic differences: *Cryptosporidium galli, Cryptosporidium baileyi* and *Cryptosporidium meleagridis*. Furthermore, some genetically distinct *Cryptosporidium* genotypes have recently been described in avian hosts (Ryan, 2010; Ryan *et al*. 2014). Among these species/genotypes, only *C. meleagridis* is of moderate public health significance due to its zoonotic and anthroponotic spread as well as its documented high infectivity rate (Wang *et al*. 2014).

Cryptosporidium is considered as an emerging pathogen in the field of avian medicine and is currently one of the most prevalent parasites affecting domestic, caged and wild birds worldwide. Infection of avian flocks with this parasite may lead to important economic losses (Pagès-Manté *et al*. 2007; Ryan and Xiao, 2008). Most studies usually focus on captive species of commercial or economic interest (Lim *et al*. 2007; Nakamura *et al*. 2009; Qi *et al*. 2011; Quah *et al*. 2011; Gomes *et al*. 2012; Papini *et al*. 2012; Wang *et al*. 2012; Baroudi *et al*. 2013; Nguyen *et al*. 2013), and fewer studies have involved wild birds (Kuhn *et al*. 2002; Papazahariadou *et al*. 2008; Yong *et al*. 2008; Abreu-Acosta *et al*. 2009; Plutzer and Tomor, 2009; Sevá *et al*. 2011). Various studies have demonstrated the presence of the zoonotic species *Cryptosporidium parvum* in avian wildlife, suggesting that birds may play a role in disseminating this parasite (Graczyk *et al*. 2008). On the other hand, studies concerning the presence and genetic identity of *Giardia* in avian hosts are scarce. Thus far, zoonotic assemblages A and B of *Giardia duodenalis* (syn. *Giardia intestinalis, Giardia lamblia*) have been found, particularly in aquatic birds (Kuhn *et al*. 2002; Majewska *et al*. 2009).

The Iberian Peninsula is particularly rich in bird life. However, no studies of the presence of *Giardia* and *Cryptosporidium* in wild birds have been carried out in this region, with the exception of a clinical case of cryptosporidiosis in Eurasian Scops-Owl (*Otus scops*) (Molina-López *et al*. 2010).

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Cryptosporidium spp. have only been described in a few studies of farm birds, such as chickens (Fernández *et al*. 1990), rheas (Ponce Gordo *et al*. 2002) and red-legged partridges (Pagès-Manté *et al*. 2007), or in ornithological gardens (Pérez Cordón *et al*. 2008). The aim of the present study was to detect and molecular characterize *Giardia* and *Cryptosporidium* in faecal samples from wild birds in Galicia (Northwest Spain).

MATERIALS AND METHODS

Sample collection and processing

Between February 2007 and September 2009, 433 faecal samples from wild birds were provided by 4 wildlife recovery centres (WRC) located in Galicia (NW Spain). The birds belonged to 64 species in 17 different orders, as follows: 138 were Accipitriformes (31·9%), 125 were Strigiformes (28·9%), 31 were Falconiformes (7·2%), 25 were Passeriformes (5·8%), 23 were Charadriiformes (5·3%), 18 were Columbiformes (4·2%), 13 were Ciconiiformes (3·0%), 13 were Pelecaniformes (3·0%), 13 were Suliformes (3·0%), 7 were Apodiformes (1·6%), 6 were Caprimulgiformes (1·4%), 5 were Procellariiformes (1·2%), 4 were Anseriformes (0·9%), 4 were Galliformes (0·9%), 4 were Gruiformes (0·9%), 3 were Piciformes (0·7%) and 1 was a Coraciiforme (0·2%) (Table 1). At the time of the admission to the WRC, the birds were isolated in individual cages. Faecal droppings were collected from the floor of the cages, stored at 4 °C and sent to the Laboratory of Parasitology, Faculty of Pharmacy, University of Santiago de Compostela for analysis. Data regarding the age, sex or health status of the animals were not provided by the WRC.

The samples (0·52 ± 0·29 g) were diluted in 10–20 ml of 0·04-M phosphate buffered saline (PBS) pH 7·2, filtered through 2 sieves (mesh size 150 and 45 µm), shaken with diethyl ether (2:1, v/v) and centrifuged at 1250 g for 15 min at 4 °C. The resulting uppermost 2 layers were carefully removed and discarded, the sediment was washed in PBS by centrifugation at 1250 g for 15 min at 4 °C, and the pellet was resuspended in 500 µl of 0·04-M PBS, pH 7·2.

Detection of Giardia *cysts and* Cryptosporidium *oocysts by epifluorescence microscopy*

A direct immunofluorescence antibody test (IFAT) was performed on 50 µl aliquots of the sediments by using the AquaGlo™ G/C Direct test (Waterborne, Inc., New Orleans, LA, USA), according to the manufacturer's instructions. The cysts/oocysts were identified by epifluorescence microscopy (400 × magnification) on the basis of their shape, size and the pattern and intensity of immunofluorescence staining. The intensity of infection by *Giardia* and/or *Cryptosporidium* was determined by counting the number of cysts/oocysts in 50 µl of concentrated sample.

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

Nucleic acids were extracted from the remaining 450 µl of sediment by using the QIAamp® DNA Stool Mini Kit (QIAGEN®, Hilden, Germany). The extraction was carried out according to the manufacturer's instructions and the DNA was stored at −20 °C until use.

A two-step nested-PCR technique was used to amplify a ∼175-bp fragment of the small subunit ribosomal gene (SSU-rDNA) and of a ∼315-bp fragment encompassing the ITS1-5·8S-ITS2 region in the ribosomal unit of *Giardia* (Read *et al*. 2002; Cacciò *et al*. 2010). For *Cryptosporidium*, a two-step nested-PCR technique was used to amplify a ∼587-bp fragment of the SSU-rDNA gene (Ryan *et al*. 2003); moreover, a protocol for amplifying a ∼325-bp fragment of the HSP-70 gene (Morgan *et al*. 2001) was tested on 8 samples. Positive and negative controls were included in all PCR experiments. The PCR products were subjected to electrophoresis on 2% agarose/ethidium bromide gels.

Positive PCR products were purified using the QIAquick® PCR Purification Kit (QIAGEN®, Hilden, Germany) and sequenced in both directions using the ABI PRISM® BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems®, Life Technologies™, Carlsbad, CA, USA) according to the manufacturer's instructions. The sequencing reactions were analysed using the ABI PRISM® 3100 automatic sequencer (Applied Biosystems®) and sequences were assembled using SeqMan™ 7·0 (DNASTAR®, Madison, WI, USA) and BioEdit 7·2·3 (©1997–2013 Tom Hall, Ibis Therapeutics, Carlsbad, CA, USA) software. The resulting sequences were compared with those deposited in GenBank® (National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, USA) using the public web interface of the BLAST® 2·2·29 program (http://blast.ncbi.nlm.nih.gov/Blast.cgi, National Centre for Biotechnology Information).

Nucleotide sequence accession numbers

Representative nucleotide sequences of the isolates analysed in this study have been deposited in the GenBank® database under accession numbers KJ939300–KJ939308.

RESULTS

Prevalence

By IFAT and PCR methods, *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts were detected in 9

Table 1. Prevalence and genotyping results for *Giardia* and *Cryptosporidium* detected in free-ranging birds in Galicia (Northwest Spain).

Scientific name (common name)	No. of samples analysed	*Giardia* Positive samples (%)	Molecular characterization (assemblages SSU-rDNA/ITS)	*Cryptosporidium* Positive samples (%)	Molecular characterization (species)
Order Accipitriformes[a]					
Family Accipitridae					
Accipiter gentilis (Northern Goshawk)	15	0	–	0	–
Accipiter nisus (Eurasian Sparrowhawk)	19	0	–	1 (5·3)	*C. parvum*
Buteo buteo (Common Buzzard)	84	1 (1·2)	A/–	7 (8·3)	*C. parvum*
Circaetus gallicus (Short-toed Snake-Eagle)	1	0	–	0	–
Circus aeruginosus(Western Marsh-Harrier)[b]	1	0	–	0	–
Circus pygargus(Montagu's Harrier)	2	0	–	0	–
Hieraaetus pennatus (Booted Eagle)	4	0	–	0	–
Milvus migrans (Black Kite)	4	0	–	1 (25·0)	*C. parvum*
Parabuteo unicinctus (Harris's Hawk)	1	0	–	1 (100)	n.d.
Pernis apivorus (Honey Buzzard)	7	0	–	1 (14·3)	*C. parvum*
Order Anseriformes[b]					
Family Anatidae					
Anas platyrhynchos (Mallard Duck)	4	2 (50·0)	F/B A/A2	2 (50·0)	n.d.
Order Apodiformes					
Family Apodidae					
Apus apus (common Swift)	6	0	–	1 (16·7)	n.d.
Apus melba (Alpine Swift)	1	0	–	0	–
Order Caprimulgiformes					
Family Caprimulgidae					
Caprimulgus europaeus (European Nightjar)	6	1 (16·7)	A/n.d.	1 (16·7)	n.d.
Order Charadriiformes					
Family Alcidae[b]					
Alca torda (Razorbill)	1	0	–	0	–
Uria aalgae (Common Guillemot)	1	0	–	0	–
Family Charadriidae					
Pluvialis apricaria (European Golden-Plover)	1	0	–	0	–
Vanellus vanellus (Northern Lapwing)	2	0	–	0	–
Family Laridae[b]					
Larus cachinnans (Caspian Gull)	6	0	–	0	–
Larus michahellis (Yellow-legged Gull)	2	0	–	0	–
Chroicocephalus ridibundus (Black-headed Gull)	2	0	–	0	–
Rissa tridactyla (Black-legged Kittiwake)	2	0	–	0	–
Family Scolopacidae					
Scolopax rusticola (Eurasian Woodcock)	3	0	–	0	–

Table 1. (*Cont.*)

Scientific name (common name)	No. of samples analysed	Giardia		Cryptosporidium	
		Positive samples (%)	Molecular characterization (assemblages SSU-rDNA/ITS)	Positive samples (%)	Molecular characterization (species)
Family Sternidae[b]					
Sterna hirundo (Common Tern)	3	0	–	0	–
Order Ciconiiformes[b]					
Family Ciconiidae					
Ciconia ciconia (White Stork)	13	0	–	1 (7·7)	n.d.
Order Columbiformes					
Family Columbidae					
Columba livia (Rock Pigeon)	2	0	–	0	–
Columba palumbus (Common Wood-Pigeon)	8	0	–	1 (12·5)	n.d.
Streptotelia decaocto (Eurasian Collared-Dove)	6	0	–	0	–
Streptotelia turtur (Turtle Dove)	2	1 (50·0)	n.d./B	1 (50·0)	*C. meleagridis*
Order Coraciiformes					
Family Upupidae					
Upupa epops (Eurasian Hoopoe)	1	0	–	0	–
Order Falconiformes[a]					
Family Falconidae					
Falco columbarius (Merlin)	1	0	–	0	–
Falco peregrinus (Peregrine Falcon)	1	0	–	0	–
Falco subbuteo (Eurasian Hobby)	7	0	–	0	–
Falco tinnunculus (Common Kestrel)	13	0	–	1 (7·7)	n.d.
Order Galliformes					
Family Phasianidae					
Alectoris rufa (Red-legged Partridge)	2	0	–	0	–
Chrysolophus pictus (Golden Pheasant)	1	0	–	0	–
Coturnix coturnix (Common Quail)	1	1 (100)	B/B	1 (100)	n.d.
Order Gruiformes[b]					
Family Rallidae					
Fulica atra (Eurasian Coot)	3	0	–	0	–
Rallus aquaticus (Water Rail)	1	0	–	0	–
Order Passeriformes					
Family Corvidae					
Corvus corone (Carrion Crow)	7	0	–	3 (42·9)	*C. parvum*
Corvus monedula (Western Jackdaw)	2	0	–	1 (50·0)	n.d.
Garrulus glandarius (Eurasian Jay)	1	1 (100)	D/n.d.	0	–
Pica pica (Eurasian Magpie)	5	1 (20·0)	n.d./B	2 (40·0)	*C. parvum*
Pyrrhocorax pyrrhocorax (Red-billed Chough)	1	0	–	0	–
Family Hirundinidae					
Delichon urbicum (Common House-Martin)	1	0	–	0	–

Ptyonoprogne rupestris (Crag Martin)	1	0	–	0	–
Family Passeridae					
Passer domesticus (House Sparrow)	2	0	–	0	–
Family Regulidae					
Regulus ignicapilla (Firecrest)	1	0	–	0	–
Family Turdidae					
Turdus merula (Eurasian Blackbird)	4	0	–	0	–
Order Pelecaniformes[b]					
Family Ardeidae					
Ardea cinerea (Grey Heron)	12	0	–	0	–
Family Phalacrocoracidae					
Phalacrocorax aristotelis (European Shag)	1	0	–	0	–
Order Piciformes					
Family Picidae					
Dendrocopos major (Great Spotted Woodpecker)	1	0	–	0	–
Picus viridis (Iberian Woodpecker)	2	0	–	0	–
Order Procellariiformes[b]					
Family Hydrobatidae					
Hydrobates pelagicus (European Storm-Petrel)	1	0	–	0	–
Family Procellariidae					
Calonectris diomedea (Cory's Shearwater)	1	0	–	0	–
Fulmarus glacialis (Northern Fulmar)	1	0	–	0	–
Puffinus puffinus (Manx Shearwater)	2	0	–	0	–
Order Strigiformes[a]					
Family Strigidae					
Asio otus (Long-eared Owl)	4	0	–	0	–
Athene noctua (Little Owl)	8	0	–	2 (25·0)	n.d.
Bubo bubo (Eurasian Eagle-Owl)	3	0	–	0	–
Otus scops (Eurasian Scops-Owl)	6	0	–	0	–
Strix aluco (Tawny Owl)	55	0	–	1 (1·8)	n.d.
Family Tytonidae					
Tyto alba (Barn Owl)	49	1 (2·0)	n.d.	6 (12·2)	n.d.
Order Suliformes[b]					
Family Sulidae					
Morus bassanus (Northern Gannet)	13	0	–	1 (7·7)	n.d.
Total	433	9 (2·1)		36 (8·3)	

[a] Raptors.
[b] Aquatic species.
n.d., not determined.

(2·1%) and 36 (8·3%) of the 433 faecal samples, respectively, being observed both pathogens in 6 samples. All positive samples contained small number of parasitic forms, i.e. 1–5 cysts/oocysts per 50 µl of concentrated faecal sample (Table 1).

Among 433 faecal samples analysed, 294 samples belonged to 20 raptor species (see Table 1). *Cryptosporidium* spp. oocysts were detected in 21 of these faecal samples (7·1%) from 9 species (45·0%): *Buteo buteo* (7/84, 8·3%), *Strix aluco* (1/55, 1·8%), *Tyto alba* (6/49, 12·2%), *Accipiter nisus* (1/19, 5·3%), *Falco tinnunculus* (1/13, 7·7%), *Athene noctua* (2/8, 25·0%), *Pernis apivorus* (1/7, 14·3%), *Milvus migrans* (1/4, 25·0%) and the sample from *Parabuteo unicinctus* (100%). *Giardia* spp. cysts were observed only in 1 sample from *B. buteo* (1·2%) and in other from *T. alba* (2·0%) (Table 1).

On the other hand, 70 (16·1%) of the faecal samples analysed in the present study belonged to aquatic species and of those, 2·8 and 5·7% were positive for *Giardia* and *Cryptosporidium*, respectively. *Cryptosporidium* spp. oocysts were detected in faecal samples from *Ciconia ciconia* (1/13, 7·7%) and *Morus bassanus* (1/13, 7·7%), and both *Cryptosporidium* and *Giardia* were detected in samples from *Anas platyrhynchos* (2/4, 50·0%) (Table 1).

In the order Passeriformes, only some species of the family Corvidae were positive (*Corvus corone, Corvus monedula, Garrulus glandarius* and *Pica pica*), showing an overall prevalence of 12·5% for *Giardia* and 37·5% for *Cryptosporidium* (Table 1).

Moreover, *Giardia* and/or *Cryptosporidium* were also detected in *Caprimulgus europaeus, Coturnix coturnix, Streptopelia turtur, Apus apus* and *Columba palumbus* (Table 1).

PCR and sequencing analyses

For *Giardia* spp., 8 samples were PCR-positive. Partial sequences of the SSU-rDNA locus revealed the presence of *G. duodenalis* assemblage A (KJ027407) in 3 samples (*A. platyrhynchos, B. buteo* and *C. europaeus*), assemblage B (JX972180) in 1 sample (*C. coturnix*), assemblage D (KJ027400) in 1 sample (*G. glandarius*) and assemblage F (AB569366) in another sample (*A. platyrhynchos*). Moreover, the results obtained for the ITS1-5·8S-ITS2 region revealed the presence of assemblage B (GU126436) in 2 more samples (*P. pica* and *S. turtur*) and confirmed those obtained for the SSU-rDNA locus, except in 1 sample (*A. platyrhynchos*) in which the sequencing results obtained for both loci were inconsistent (Table 1).

With respect to *Cryptosporidium* spp., partial sequences of the SSU-rDNA and HSP70 genes were obtained in 4 and 3 isolates, respectively. Sequencing analyses revealed that 6 sequences obtained from *A. nisus, B. buteo, C. corone,* *M. migrans* and *P. apivorus*, were identical to sequences of the SSU-rDNA and HSP70 genes of *C. parvum* deposited in GenBank® (KJ569798), and another one (*S. turtur*) was identical to sequence KC734572 corresponding to *C. meleagridis* (Table 1).

DISCUSSION

This is one of the largest parasitological studies involving detection of *Giardia* and *Cryptosporidium* carried out on wild birds worldwide and the first performed in the Iberian Peninsula, covering 42% of the bird species described in the region of Galicia (approximately 152 bird species are known to inhabit this area) (Anonymous, 2014). Little is known about the presence of *Giardia* and/or *Cryptosporidium* in wild birds. Most previous studies have focused on species that play a potential role in the mechanical transmission of these entero-pathogens, such as aquatic and migratory birds (Kuhn *et al*. 2002; Graczyk *et al*. 2008; Majewska *et al*. 2009; Plutzer and Tomor, 2009).

The overall prevalence of *Giardia* in the samples was 2·1%, as determined by IFAT and PCR analysis. This value is lower than in previous studies carried out in several species of wild birds, in which the prevalence ranged from 5·0 to 28·0% (Kuhn *et al*. 2002; Papazahariadou *et al*. 2008; Majewska *et al*. 2009; Plutzer and Tomor, 2009). The prevalence of *Cryptosporidium* was 8·3%, which is consistent with the data reported in the international literature: 5·8% in Hungary (Plutzer and Tomor, 2009), 6·3% in Australia (Ng *et al*. 2006), 7·2% in USA (Ziegler *et al*. 2007) and 13·0% in Greece (Papazahariadou *et al*. 2008).

Most of the faecal samples examined in the present study corresponded to raptor species (68·0%). The prevalence of *Giardia* in these species was 0·7% and that of *Cryptosporidium*, 7·1%. To our knowledge, *Giardia* spp. is described for the first time in the Barn Owl (*T. alba*) and *G. duodenalis* assemblage B in the Common Buzzard (*B. buteo*). Likewise, *Cryptosporidium* spp. is described for the first time in the Tawny Owl (*S. aluco*), Little Owl (*A. noctua*), Common Kestrel (*F. tinnunculus*) and Harris Hawk (*P. unicinctus*). In addition, *C. parvum* was found for the first time in the Common Buzzard (*B. buteo*), Sparrowhawk (*A. nisus*), Honey Buzzard (*P. apivorus*) and Black Kite (*M. migrans*).

Raptors are located at the top of the food chain and detection of both parasite species, especially *Cryptosporidium*, in this group indicates that the parasites are present in the environment. Food sources play a major role in the transmission of cryptosporidiosis in birds of prey, because most raptor species feed on small mammals, other birds or fish and reptiles, which may be infected (Ryan, 2010).

With respect to aquatic species, *Cryptosporidium* spp. oocysts were observed in one faecal sample from a White Stork (*C. ciconia*). Both *C. parvum* and *G. duodenalis* have been found in this host species in Poland (Majewska *et al*. 2009). *Cryptosporidium* spp. oocysts were also detected in a sample of a Northern Gannet (*Morus bassanus*) in the present study, which as far as we know constitutes the first description of *Cryptosporidium* in this species and in the order Suliformes.

Moreover, *G. duodenalis* assemblages A and B cysts and *Cryptosporidium* sp. oocysts were detected in 2 of the 4 samples of Mallard Duck (*A. platyrhynchos*), suggesting that there is a high chance that this species will act as a carrier of these pathogens (Majewska *et al*. 2009; Plutzer and Tomor, 2009). These cases of co-detection are according to the findings made by other authors (Kuhn *et al*. 2002; Plutzer and Tomor, 2009). Ducks are more likely than other birds to become infected from water contaminated with faecal material of human or domestic animal origin, because of their strict aquatic habit (Cacciò *et al*. 2005).

Environmental contamination with human and domestic-animal faecal material is recognized as a potential pathogen pathway for wildlife infections with zooanthroponotic parasites such as *Giardia* and *Cryptosporidium* (Appelbee *et al*. 2005; Kutz *et al*. 2009; Thompson, 2013). Indeed, previous studies of environmental samples, wild otters, bivalve molluscs and freshwater macroinvertebrates in Galicia provided evidence that streams in this region are widely contaminated with *Giardia* and *Cryptosporidium* (Gómez-Couso *et al*. 2005, 2006; Méndez-Hermida *et al*. 2007; Reboredo-Fernández *et al*. 2014). Moreover, the rate of contamination with these enteropathogens in Galician water bodies has been positively correlated with levels of livestock activity (Castro-Hermida *et al*. 2010). As livestock constitute a potential source of environmental contamination with these pathogens, other animals that live on or close to farms are at risk of being infected (Ziegler *et al*. 2007; Castro-Hermida *et al*. 2009; Gracenea *et al*. 2011). Moreover, different studies have shown that invertebrates such as snails can act as bioindicators of soil contamination (Neira *et al*. 2010) and that non-biting synanthropic flies can transport infectious oocysts of *C. parvum* in their digestive tracts and/or on external surfaces in a transmission route that may occur in rural areas (Clavel *et al*. 2002; Conn *et al*. 2007).

As it has mentioned previously, in birds belonging to the order Passeriformes, all the positive samples were included in the family Corvidae. The prevalence of *Cryptosporidium* was 37·5% in Corvidae, which is very similar to the prevalence of 33·9% reported for these birds in Malaysia (Yong *et al*. 2008). In the same way, the prevalence of *Giardia* (13·0%) is also relatively high for this family of birds. Moreover, zoonotic genotypes/species of both parasites were identified in these hosts. Thus, *C. parvum* was identified in a Carrion Crow (*C. corone*) and in a Eurasian Magpie (*P. pica*). *C. parvum* has been reported in *C. corone* in Poland (Majewska *et al*. 2009); however, to our knowledge, this is the first time that *C. parvum* has been detected in *P. pica*, although *C. baileyi* has been described in this species in China (Qi *et al*. 2011). *G. duodenalis* assemblage B was found in the Eurasian Magpie (*P. pica*) constituting the first description of this species/assemblage of *Giardia* in this avian host. Also, *G. duodenalis* assemblage D (canine genotype) was identified in the faecal sample from a Eurasian Jay (*G. glandarius*). This is the first time that this assemblage of *Giardia* has been detected in birds. Corvids are widely distributed and are very common in urban landscape, and they may therefore constitute an important source of transmission of the parasite forms (Yong *et al*. 2008).

Interestingly, some other species were positive for *Giardia* and/or *Cryptosporidium*. *G. duodenalis* assemblage B and *Cryptosporidium* sp. oocysts were detected in a faecal sample from Common Quail (*C. coturnix*). This is the first time that *G. duodenalis* assemblage B has been found in this host. However, *Cryptosporidium* was previously reported in a study carried out in farm quails in China, in which *C. baileyi* was more prevalent than *C. meleagridis* (Wang *et al*. 2012). A faecal sample from the European Nightjar (*C. europaeus*) contained cysts of the zoonotic *G. duodenalis* assemblage A as well *Cryptosporidium* sp. oocysts. Moreover, *Cryptosporidium* sp. oocysts were also found in Common Swift (*A. apus*). To our knowledge, this the first report of *Giardia* in the order Caprimulgiformes and of *Cryptosporidium* in the orders Apodiformes and Caprimulgiformes.

The presence of *C. meleagridis* in the Turtle Dove (*S. turtur*) extends the range of this enteropathogen in avian hosts and raises questions about the potential zoonotic transmission of cryptosporidiosis from doves to humans, as *C. meleagridis* is an emerging human pathogen and constitutes the third most common *Cryptosporidium* parasite in humans in some geographical locations (McLauchlin *et al*. 2000; Matos *et al*. 2004; Cama *et al*. 2008).

Birds are important components of the ecosystem because they act as pollinators, agents of seed dispersal and natural pest controllers, as well as scavengers and environmental cleaners. They are therefore often used as environmental health indicators (Carignan and Villard, 2002; Gregory and Strien, 2010). Moreover, anthropogenic alterations to the environment caused by modern agricultural practices and urban sprawl may impact wildlife health through stress and exposure to introduced pathogens (Daszak *et al*. 2001; Kutz *et al*. 2009). A better understanding of how bird populations may

be affected by infections and the potential impact that pathogens have on wild populations is therefore required. Finally, the results obtained in this study demonstrate the wide dissemination of *Giardia* and/or *Cryptosporidium* in wild birds. Further studies are needed to confirm whether the presence of the cysts/oocysts in the wild bird species is due to mechanical transport or to a true infection.

CONFLICT OF INTEREST

None of the authors have any commitments, consultancies or contracts that could be considered as conflicts of interest with respect to this study.

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