

The role of free-ranging, captive, and domestic birds of Western Poland in environmental contamination with *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts

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Abstract As *Cryptosporidium parvum* and *Giardia lamblia* can be disseminated in the environment by avian hosts, a total of 499 fecal dropping from 308 free-ranging, 90 captive, and 101 domestic birds were tested by conventional, immunological, and molecular techniques for these human enteropathogens. Twenty-six (5.2%) tested positive for *G. lamblia* cysts and 19 (3.8%) for *C. parvum* oocysts. A bird total of 23 (7.5%) free-ranging, two (2.2%) captive, and one (0.1%) domestic tested positive for cysts, whereas 18 (5.8%) free-ranging, one (1.1%) captive, and zero livestock birds tested positive for oocysts. *G. lamblia* cysts and *C. parvum* oocysts were found significantly more frequently in fecal droppings of free-ranging aquatic birds than in birds not normally associated with water. No specimen tested positive for both pathogens simultaneously. Aquatic birds represent an important epidemiologic link in water-associated transmission

cycles of *Cryptosporidium* and *Giardia* and play a significant role in environmental contamination of aquatic habitats with these anthrozoönotic pathogens.

Introduction

Cryptosporidium and *Giardia* are environmentally ubiquitous enteropathogens that cause serious human and animal diseases, which are frequently of waterborne etiology. The presence of *Cryptosporidium* oocysts and *Giardia* cysts in source waters represents a serious public health threat as these pathogens have caused numerous outbreaks related to drinking and recreational waters (Karanis et al. 2007). *Cryptosporidium* and *Giardia* infections cause considerable economic losses in livestock production (Olson et al. 2004).

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There are many point and non-point sources of environmental contamination of aquatic habitats with these enteropathogens. Wastewater discharges into surface waters can cause an accumulation of *Cryptosporidium* and *Giardia* in potable waters and negatively impact on drinking water quality and consequently the public health (Rose et al. 2002; Karanis et al. 2007). As *Cryptosporidium* and *Giardia* have an extensive zoonotic reservoir, particularly associated with aquatic habitats, a variety of animals can propagate and disseminate oocysts and cysts, respectively, in the environment (Majewska et al. 1997, 1998; Solarczyk and Majewska, 2007; Graczyk et al. 2008). A broad variety of free-ranging, captive, and domestic bird species can excrete human-virulent pathogens in their fecal droppings (Graczyk et al. 2008). These pathogens include bacteria (Buck 1990; Kirschner et al. 2004; Heuvelink et al. 2008); fungi, e.g., microsporidia (Haro et al. 2005, 2006; Lobo et al. 2006; Slodkiewicz-Kowalska et al. 2006; Graczyk et al. 2007b); and protozoa, i.e., *Giardia* and *Cryptosporidium*, (Graczyk et al. 1998; Kuhn et al. 2002; Zhou et al. 2004; Jellinson et al. 2004, 2007; Heuvelink et al. 2008). However, the role of birds as polluters of aquatic habitats with *Cryptosporidium* oocysts and *Giardia* cysts has rarely been investigated (Graczyk et al. 2008). Drinking water sources or recreational waters are not routinely monitored for these pathogens, and considerable evidence demonstrates their direct zoonotic association with birds, including waterfowl species (Graczyk et al. 2008).

The vectorial capacity of waterfowl for delivering *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts to surface water is influenced by a set of host-related and pathogen-specific factors that result in the establishment of infection in birds (i.e., the reservoir host) or the lack of infection (i.e., mechanical passage; Graczyk et al. 2008). As documented by laboratory and field experiments, *C. parvum* oocysts can be mechanically passed to water by birds (Graczyk et al. 1996, 1997, 1998; Zhou et al. 2004). Migratory Canada geese (*Branta canadensis*) frequently follow cattle (i.e., reservoir host of *C. parvum*) at their stop-over places and take advantage of undigested plant material in cattle feces (Graczyk et al. 1998) and, thus, acquire *C. parvum* oocysts via ingestion. Residential Canada geese have also ingested *Cryptosporidium hominis* oocysts by scavenging garbage (Zhou et al. 2004). As *C. hominis* cycles predominantly among people, it is reasonable to assume that the oocysts of this species found in goose fecal droppings (Zhou et al. 2004) indicate a mechanical transfer rather than an indigenous *C. hominis* infection. *Giardia* have a wide zoonotic reservoir (Wolfe 1992; Majewska and Kasprzak 1990, 2000), with some avian isolates of cysts virulent to mammals (Upcroft et al. 1997, 1998), indicating that birds can serve as a reservoir host in addition to being a mechanical vector (Graczyk et al. 1998; Zhou et al. 2004).

The purpose of the present study was to determine the prevalence of free-ranging, captive, and domestic birds of Western Poland that shed *C. parvum* oocysts *G. lamblia* cysts in their fecal droppings.

Materials and methods

A total of 499 specimens of fecal dropping were collected from 308 free-ranging, 90 captive, and 101 domestic birds (Table 1). The birds represent 39 species belonging to ten orders and 13 families (Table 1). Specimens of fecal droppings originated from 11 sites in Western Poland (Fig. 1). Fecal droppings of free-ranging birds were collected at seven sites, including the Warta Mouth National Park (Site 1) and the Great Bytyń Lake Nature Reserve (Site 2; Fig. 1). Specimens from captive birds were collected in the Poznan Zoological Garden (Site 8; Fig. 1). Domestic bird samples were collected from a commercial farm (Site 9) and two private farms (Sites 10 and 11; Fig. 1). Specimens from free-ranging birds were obtained during ornithological observations or capture-and-release ringing, whereas samples from captive and domestic birds were collected from the ground of aviaries or the floor of birdcages and from poultry houses, respectively. Fresh fecal material from an individual bird was placed separately into a plastic tube, labeled, and transported to the laboratory in a cooler.

A direct wet smear was prepared from each of specimen in triplicate by mixing a small amount of stools, i.e., approximately 2 g, with 0.1 ml of phosphate-buffered saline (PBS) pH 7.4. A drop of Lugol's iodine (Ash and Orihel 1987) was added while the smears were still wet to the first set of slides, the slides were coverslipped, and the entire coverslipped area was examined under $\times 60$ microscope objective magnification. For detection of *Giardia* cysts, the second set of smears was fixed in Schaudinn's fixative and then stained with iron hematoxylin (Ash and Orihel 1987). For demonstration of *Cryptosporidium* oocysts, the smears were dried, fixed with methanol, and stained with Ziehl-Neelsen (Ash and Orihel 1987). Smears stained with iron hematoxylin and Ziehl-Neelsen were examined using the oil-immersion objective, i.e., $\times 100$.

The commercial test kit (ProSpecT[®] *Cryptosporidium* [Monoclonal] Microplate Assay, Remel, Dartford, Kent, UK) was used according to the manufacturer's instruction to detect *Cryptosporidium* coproantigen in all bird fecal droppings.

To confirm identification of *C. parvum* oocysts and *G. lamblia* cysts, all positive specimens confirmed by the aforementioned methods and several randomly selected specimens were re-tested using multiplexed fluorescence in situ hybridization (FISH) combined with immunofluorescent

Table 1 List of bird species examined for the presence of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts in their fecal droppings

Order	Family	Species (no. of specimens)		
Anseriformes	Anatidae	Mallard duck, <i>Anas platyrhynchos</i> (32) ^a		
		Greylag goose, <i>Anser anser</i> (34) ^a		
		Common merganser, <i>Mergus merganser</i> (72) ^a		
		Mute swan, <i>Cygnus olor</i> (32) ^a (1) ^b		
		Black swan, <i>Cygnus atratus</i> (4) ^b		
		Black-necked swan, <i>Cygnus melancoryphus</i> (4) ^b		
		Coscoroba swan, <i>Coscoroba coscoroba</i> (1) ^b		
		Emperor goose, <i>Chen canagica</i> (1) ^b		
		Canada goose, <i>Branta canadensis</i> (18) ^b		
		Red-breasted goose, <i>Branta ruficollis</i> (2) ^b		
		Common shelduck, <i>Tadorna tadorna</i> (1) ^b		
		Mandarin duck, <i>Aix galericulata</i> (3) ^b		
		Domestic goose, <i>Anser anser f. domestica</i> (11) ^c		
		Ciconiiformes	Ardeidae	Grey heron, <i>Ardea cinerea</i> (2) ^a
Ciconiidae	White stork, <i>Ciconia ciconia</i> (16) ^a (8) ^b			
	Black stork, <i>Ciconia nigra</i> (4) ^b			
	Marabou stork, <i>Leptoptilos crumeniferus</i> (2) ^b			
Phoenicopteriformes	Threskiornithidae	Sacred ibis, <i>Threskiornis aethiopicus</i> (2) ^b		
	Phoenicopteridae	Andean flamingo, <i>Phoenicopeterus andinus</i> (3) ^b		
Passeriformes	Corvidae	Carrion crow, <i>Corvus cornix</i> (63) ^a		
Psittaciformes	Psittacidae	Rook, <i>Corvus frugilegus</i> (57) ^a		
		Budgerigar, <i>Melopsittacus undulatus</i> (1) ^b		
		Rose-ringed parakeet, <i>Psittacula krameri</i> (2) ^b		
		African grey parrot, <i>Psittacus erithacus</i> (4) ^b		
		Masked lovebird, <i>Agapornis personata</i> (1) ^b		
		Blue-and-yellow macaw, <i>Ara ararauna</i> (2) ^b		
		Orange-winged parrot, <i>Amazona amazonica</i> (2) ^b		
		Cuban parrot, <i>Amazona leucocephala</i> (1) ^b		
		Amazona brillante, <i>Amazona autumnalis</i> (3) ^b		
		Eastern rosella, <i>Platycercus eximius</i> , (1) ^b		
		Cockatiel, <i>Nymphicus hollandicus</i> (1) ^b		
		Piciformes	Cacatuidae	Toco toucan, <i>Ramphastos toco</i> (1) ^b
			Rhamphastidae	Trumpeter hornbill, <i>Bycanistes bucinator</i> (2) ^b
		Bucerotiformes	Bucerotidae	Crane, <i>Grus grus</i> (3) ^b
Gruiformes	Gruidae	Black crowned crane, <i>Balearica pavonina</i> (4) ^b		
Pelecaniformes	Pelecanidae	White pelican, <i>Pelecanus onocrotalus</i> (7) ^b		
Galliformes	Phasianidae	Indian peafowl, <i>Pavo cristatus</i> (1) ^b		
		Domestic chickens (40) ^c		
		Turkey (50) ^c		

^a Free-ranging birds^b Captive birds^c Domestic birds

antibody (IFA; Graczyk et al. 2007a). FISH oligonucleotide probes were synthesized by the DNA Analysis Facility of the Johns Hopkins University, Baltimore, MD, USA, in 1.0- μ M scale, purified by HPLC, and 5'-labeled with a single molecule of a fluorochrome, hexachlorinated 6-carboxyfluorescein (Graczyk et al. 2007a). A FITC-conjugated monoclonal IFA against the cell wall antigens of *Cryptosporidium* and *Giardia* from MERIFLUOR™ *Cryptosporidium/Giardia* test kit (Meridian Diagnostic, Cincinnati, OH, USA) was used (Graczyk et al. 2007a). The walls of the pathogen's transmissive stages were permeabilized (Graczyk et al. 2007a). All combined FISH and direct IFA reactions were carried out in Eppendorf tubes in a total volume of 100 μ l of hybridization buffer at 48°C for 1 h (Graczyk et al. 2007a). The concentration of

each oligonucleotide probe, i.e., CRY-1, GIAR-4, and GIAR-6 (Graczyk et al. 2007a) was 1 mMol l⁻¹ and IFA was 1:1 diluted. Positive and negative controls were prepared as previously described (Graczyk et al. 2007a). After hybridization, the tubes were centrifuged twice at 4°C (2,000 \times g, 5 min), and the pellets were resuspended in 100 μ l of sterile PBS. Five, 20 μ l samples were transferred onto lysine-coated wells (5 mm diameter) on a Teflon-coated glass slide and air-dried. The entire area of a well was examined with the aid of an Olympus BH2-RFL epifluorescent microscope, dry \times 60 objective, and BP450-490 exciter filter without knowledge of sample identity, the pathogens were enumerated and the samples un-coded.

Statistical analysis of *Giardia* and *Cryptosporidium* association in fecal droppings of free-ranging, captive, and

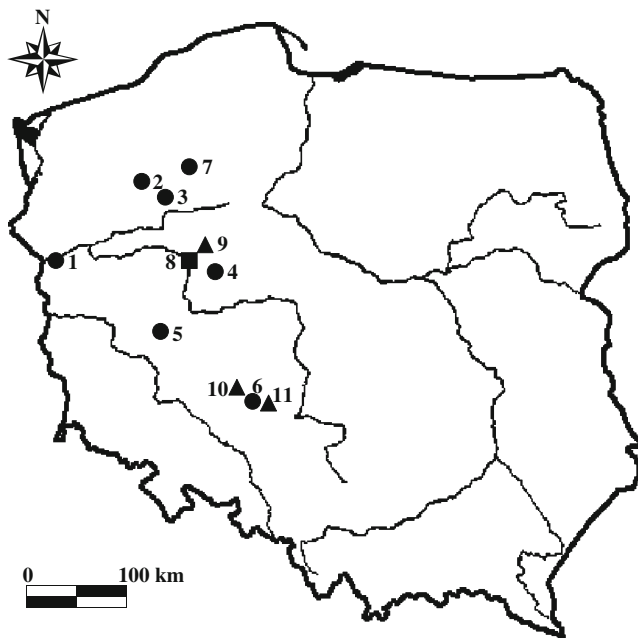


Fig. 1 Collection sites of bird fecal droppings. • Free-ranging birds: 1 Warta Mouth National Park, 2 The Wielki Bytyń Lake Nature Reserve, 3 Piła, 4 Iwno, 5 Leszno, 6 Kępno, 7 Złotów. ■ Captive birds: 8 the Poznań ZOO. ▲ Domestic birds: 9 Koziegłowy, 10 Kliny, 11 Wieruszów

domestic birds was carried out using Chi-square (χ^2) test. Statistical significance was defined as P value <0.05 .

Results

Overall, *Cryptosporidium* oocysts and *Giardia* cysts were detected in bird fecal droppings at five of 11 (46%) and four of 11 (36%) sites, respectively. Fecal droppings positive for *C. parvum* oocysts originated from the Warta Mouth National Park (Site 1), Great Bytyn Lake Nature Reserve (Site 2), Leszno (Site 5), Kępno (Site 6), and the Poznan Zoological Garden (Site 8; Fig. 1). *G. lamblia*-positive samples originated from the Warta Mouth National Park (Site 1), Great Bytyn Lake Nature Reserve (Site 2), Poznan Zoological Garden (Site 8), and Wieruszów (Site 11; Fig. 1). Of the 499 samples, 26 (5.2%) tested positive for *G. lamblia* cysts and 19 (3.8%) for *C. parvum* oocysts by multiplexed FISH combined with IFA method. A total 23 (7.5%) free-ranging, two (2.2%) captive, and one (0.1%) domestic bird were positive for *G. lamblia* cysts, whereas 18 (5.8%) free-ranging, one (1.1%) captive, and none of livestock birds tested positive for *C. parvum* oocysts by multiplexed FISH combined with IFA method. *G. lamblia* cysts and *C. parvum* oocysts were found significantly more frequently ($\chi^2=9.49$, $P<0.010$) in fecal droppings of aquatic birds (Tables 2 and 3) than in birds not associated with water.

Table 2 Results of testing of bird fecal droppings for *Giardia lamblia* cysts by the multiplexed fluorescence in situ hybridization (FISH) method in combination with immunofluorescent antibody (IFA)

Bird species	Samples		
	Total no.	Nos. of positive	% positive
Mallard duck (<i>Anas platyrhynchos</i>) ^a	32	7	21.9
Greyleg goose (<i>Anser anser</i>) ^a	34	10	29.4
Black crowned crane (<i>Balearica pavonina</i>) ^b	4	1	25
Common merganser (<i>Mergus merganser</i>) ^a	72	1	1.4
Mute swan (<i>Cygnus olor</i>) ^a	33	4	12.5
Domestic goose (<i>Anser anser f. domestica</i>) ^c	11	1	9.1
White stork (<i>Ciconia ciconia</i>) ^d	24	1	4.2
Carrion crow (<i>Corvus corone</i>) ^a	63	1	1.6

^a Free-ranging birds

^b Captive birds

^c Domestic birds

^d Sixteen samples from free-ranging and eight from captive birds

The prevalence of fecal droppings positive for *C. parvum* oocysts and *G. lamblia* cysts varied significantly among the three groups of birds ($\chi^2=9.27$, $P<0.010$; $\chi^2=8.45$; $P<0.015$, respectively). The prevalence of *C. parvum*- and *G. lamblia*-positive fecal specimens was significantly higher in free-ranging (5.8% and 7.5%, respectively) than in captive (1.1% and 2.2%, respectively) or domestic birds (0% and 0.99%, respectively; $\chi^2=8.01$, $P<0.018$). No specimen tested positive for both pathogens simultaneously.

The three diagnostic techniques, i.e., wet fecal, hematoxylin-stained smears, and FISH combined with IFA) had

Table 3 Results of testing of bird fecal droppings for *Cryptosporidium parvum* oocysts by the multiplexed fluorescence in situ hybridization (FISH) method in combination with immunofluorescent antibody (IFA)

Bird species	Samples		
	Total no.	Nos. of positive	% positive
Mandarin duck (<i>Aix galericulata</i>) ^a	3	1	33.3
Common merganser (<i>Mergus merganser</i>) ^b	72	2	2.8
Mute swan (<i>Cygnus olor</i>) ^b	33	4	12.5
White stork (<i>Ciconia ciconia</i>) ^c	24	3	12.5
Carrion crow (<i>Corvus corone</i>) ^a	63	6	9.5
Rook (<i>Corvus frugilegus</i>) ^b	57	3	5.3

^a Captive birds

^b Free-ranging birds

^c Sixteen samples from wild and eight from captive birds

the same sensitivity for identification of *Giardia* cysts. *G. lamblia* cysts were identified in droppings of free-ranging birds such as Greylag geese, Mallard ducks, Mute swans, and Carrion crows living in the Warta Mouth National Park (Site 1) and from Common mergansers in the Great Bytyn Lake Nature Reserve (Site 2; Fig. 1; Table 2). Fecal dropping specimens of a White stork and Black crowned crane in Poznan Zoological Garden (Site 8) as well as of domestic goose from a private farm (Site 11) were positive for *G. lamblia* cysts (Fig. 1). In all *Giardia*-positive fecal droppings, a small number of cysts, i.e., one to five per slide, were detected.

Sensitivity of FISH in detection of *C. parvum* oocyst was only slightly higher than by Ziehl–Neelsen and immunoassay. The oocysts were predominantly identified in fecal droppings of aquatic free-ranging birds (Table 3). The oocysts were detected by FISH in combination with IFA and by Ziehl–Neelsen in droppings from six Carrion crows and three Mute swans in the Warta Mouth National Park (Site 1; Fig. 1; Table 3); *Cryptosporidium* coproantigen was also present in these samples. Also, one of nine (11%) of Mute swans from the Great Bytyn Lake Nature Reserve (Site 2) was positive for *C. parvum* oocysts and *Cryptosporidium* coproantigen. Only two of 72 (3.8%) Common mergansers from the same location were positive for *C. parvum* oocysts solely by FISH in combination with IFA. *C. parvum* oocysts and *Cryptosporidium* coproantigen were found in fecal droppings of three White storks (Site 5; Fig. 1) and three Rooks (Site 6 (Fig. 1). *C. parvum* oocysts were identified in one Mandarin duck fecal specimen from the Poznan Zoological Garden (Site 8; Fig. 1); only a single oocyst was identified by multiplexed FISH in combination with IFA. In all *C. parvum*-positive samples, the number of oocysts ranged from one to eight per slide.

Discussion

Studies on birds disseminating human-virulent species of *Cryptosporidium* oocysts or *Giardia* cysts are scant (Graczyk et al. 2008) and predominantly relate to North America (Graczyk et al. 1998; Zhou et al. 2004) and Europe (Heuvelink et al. 2008). The present study demonstrated that aquatic birds are involved in water-associated transmission cycles of *C. parvum* and *G. lamblia* as the oocysts and cysts were predominantly identified in fecal droppings of aquatic, free-ranging birds (Table 2 and 3). Although this confirms the previous findings (Graczyk et al. 1998, 2008; Kuhn et al. 2002; Kassa et al. 2004; Zhou et al. 2004; Jellinson et al. 2004, 2007; Heuvelink et al. 2008), the concentration of *C. parvum* oocysts and *G. lamblia* cysts in bird droppings in the present study was much lower than observed previously (Graczyk et al. 1998, Kuhn et al. 2002). This indicates a

mechanical passage of oocysts and cysts rather than indigenous infections. The premise that birds are being infected with human infective *Cryptosporidium* spp. or *Giardia* spp. cannot be substantiated by the results of this study. However, indigenous giardiasis cannot be completely excluded as a *Giardia* isolate belonging to the *Giardia duodenalis* group was described in free-ranging sulfur-crested cockatoos (Upcroft et al. 1997, 1998). This particular isolate was lethal to birds and pathogenic to mice (Upcroft et al. 1997, 1998). We conclude that aquatic birds represent an important epidemiologic link in water-associated transmission cycles of *Cryptosporidium* and *Giardia* and also play a significant role in environmental contamination of aquatic habitats with these anthroozoonotic pathogens. Prevention of such contamination is technologically and logistically difficult, because waterfowls (1) can migrate long distances, (2) usually occur in large flocks, (3) have unlimited access to surface water and frequently graze and defecate directly into water or on the banks of water reservoirs, and (4) are protected by environmental law in many regions.

Reported prevalence of birds shedding *C. parvum* oocysts or *G. lamblia* cysts varies considerably (Graczyk et al. 2008) and can be as high as 90% (Kassa et al. 2004). Concentration of infectious *C. parvum* oocysts (as per mouse bio-assay) and *Giardia* sp. cysts in fecal droppings of migratory Canada geese was 3.7×10^3 and 4.1×10^3 /g, respectively (Graczyk et al. 1998), and 4.8×10^2 and 4.4×10^4 /g, respectively, in migratory ducks (Kuhn et al. 2002). Prediction models developed on the basis of field data (Kirschner et al. 2004) indicated that a single visitation of an average size waterfowl flock can introduce into the water approximately 9.3×10^6 infectious *C. parvum* oocysts and 1.1×10^7 *G. lamblia* cysts into the water (Graczyk et al. 2008). Such a high number of transmissive stages of these enteropathogens represent a serious public health threat. *C. parvum* isolates differ in their virulence to humans (Okhuysen et al. 1999; Messner et al. 2001). Volunteer challenge trials with healthy *C. parvum*-seronegative individuals demonstrated that the ID₅₀ for the three *C. parvum* isolates (i.e., TAMU, IOWA, and UCP) varied from nine to 18, 87 to 190, and 1,042 to 2,980 oocysts, respectively (Okhuysen et al. 1999; Messner et al. 2001). Giardiasis in immunocompetent people can be caused by only ten cysts (Wolfe 1992).

A majority of bird droppings containing *C. parvum* oocysts and *G. lamblia* cysts originated from free-ranging birds from the Warta Mouth National Park (Site 1) and the Great Bytyn Lake Nature Reserve (Site 2; Fig. 1). These are protected wetlands, which feed multiple river tributaries from which water is abstracted for drinking water processing. As public access to these wetlands is limited, the possibility of acquiring cryptosporidiosis or giardiasis via direct contact is low. However, this is still plausible as some

parts of these parks are open for water recreation, ecotourism, and ecological and environmental research. In water, both oocysts and cysts retain their infectivity for a prolonged period; from 2 months for *Giardia* (Wolfe 1992) and up to a year for *Cryptosporidium* (Tamburrini and Pozio 1999); this considerably facilitates transmission via recreational water contact. In addition, their small sizes and resistance to standard water chlorination (e.g., oocysts) facilitate transmission via drinking water. These two national parks cumulate extraordinary high number, i.e., up to 10^5 birds; thus, their input of human-virulent oocysts and cysts to the environment is substantial, despite the fact that the oocyst and cyst concentration was low. We conclude that *C. parvum* and *G. lamblia* transmission cycles related to water can be easily sustained in these parks as they are densely populated by semiaquatic and aquatic mammals, i.e. Eurasian otter, beavers, Red fox, Raccoon dog, Eurasian badger, Least weasel, Stoat, Beach marten, Pine marten, European polecat, and American Mink. Recently, *Cryptosporidium* oocysts and *Giardia* cysts were found in fecal samples of free-ranging otters in northwest Spain (Méndez-Hermida et al. 2007).

Finding of human-virulent *C. parvum* oocysts and *G. lamblia* cysts in fecal droppings of Carrion crows and Rooks (i.e., corvids) indicates that these synanthropic species represent a real zoonotic threat to people, predominantly via direct encounters. Corvids are abundant near human settlements and can substantially contribute to the contamination of areas shared with people. In the present study, over 12% of free-ranging White storks tested positive for *C. parvum* (Table 3). White storks are also synanthropic birds that nests near human settlements, often graze at meadows, pastures, wetlands, and other kinds of surface water reservoirs shared with people and travel long distances. Interestingly, a study carried out in Europe demonstrated that *Campylobacter* spp. were found in 13% of corvids and meadow birds (Heuvelink et al. 2008).

For identification of viable pathogens such as *C. parvum* and *G. lamblia*, FISH is more advantageous than PCR because it allows species-specific identification, visualization, and viability assessment of up to a single pathogen cell. Such resolution is not available or extremely impractical with any other technique. For example, using highly sensitive RT-PCR, the lowest number of *C. parvum* oocysts that can be assessed for viability was 10^3 (Jenkins et al. 2000). Considering the advantages of FISH for identification of viable pathogens of medical and veterinary importance, it is surprising that this technique has not been widely implemented into screening of a variety of environmental samples. In addition, as only weak autofluorescence of nonstructural debris was observed in the present study, FISH is a most suitable technique for identification of human pathogens in bird fecal droppings.

Further studies on the role of aquatic birds in disseminating human-virulent *Cryptosporidium* oocysts and *Giardia* cysts are needed with the involvement of molecular epidemiologists, ornithologists, veterinarians, and clinicians. Also, molecular genotyping and source-tracking of oocysts and cysts originating from birds is necessary to establish their true epidemiologic significance and public health importance. Advances in molecular technology make source-tracking determinations and genetic analysis possible. The determination of genetic diversity of bird-specific *Cryptosporidium* is essential as novel *Cryptosporidium* “goose” and “duck” genotypes (Jellinson et al. 2004, 2007; Zhou et al 2004) have not been seen in humans, and human virulence of *Cryptosporidium* spp. oocysts found in 90% of goose or 49% duck droppings (Kuhn et al. 2002; Kassa et al. 2004) is unknown.

The aquatic environment is a resource shared by people and wildlife, and their environmental and ecological interactions will continue. Considerable evidence indicates that migratory birds can contribute to the global spread of infectious agents in both a temporal and spatial manner. Waterfowls are considered part of our natural resource heritage and are necessary for ecological health and the proper functioning of aquatic ecosystems. Therefore, in the areas where their presence is environmentally justifiable, these birds should be supported and protected.

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