

The role of birds in dissemination of human waterborne enteropathogens

Thaddeus K. Graczyk^{1,2,3}, Anna C. Majewska⁴ and Kellogg J. Schwab^{1,2,3}

¹ Department of Environmental Health Sciences, Division of Environmental Health Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD3 21205, USA

² Johns Hopkins Center for Water and Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA

³ Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA

⁴ Department of Biology and Medical Parasitology, Poznan University of Medical Sciences, 61-701 Poznan, Poland

Cryptosporidiosis, giardiasis and microsporidiosis are serious human diseases of waterborne origin; their etiologic agents and a substantial fecal coliform load can enter surface, drinking and recreational water resources from aquatic birds. The aim of this article is to present interactions between waterfowl and these waters that imply a negative public health impact, reinforcing the need for either better water-quality indicators or for water monitoring specifically for *Cryptosporidium*, *Giardia* and microsporidia. Where justifiable, the presence of waterfowl should be supported; however, management of drinking and recreational water resources needs to be improved by incorporating effective protection measures for pathogens linked to these birds.

Waterfowl and standard water-quality parameters

From the drinking water production standpoint, the presence of aquatic birds at water reservoirs is associated with steadily decreasing water quality [1,2]. Birds residing at waters used for body-contact recreation have been responsible for the deterioration of microbiological quality of this water [3]. Waterfowl contribute a substantial amount of fecal indicators to water sources [2–4], the quantitative measurement of which is commonly used as standard water-quality parameters (i.e. enterococci, *E. coli*, fecal coliforms or total coliforms). The early recognition of non-point sources of coliform contribution by birds [3,4] has prompted fecal coliform source tracking efforts [5,6]. However, because of the level of technology required (which has not currently been developed) [5,6], this methodology is not feasible for drinking water utilities or environmental laboratories. Instead, fecal coliform source tracking is used as a research tool for watershed protection purposes [6] or for characterization of the antibiotic-resistance of coliforms from waterfowl [5]. Drinking water production processes can be adjusted according to fecal coliform levels in source waters without knowledge of their origin. There is a wide variety of disinfection technologies for dealing with source water of low microbiological quality, thus making fecal coliforms at the drinking water source level an ‘engineering’ rather than an ‘environmental microbiology’ issue. Herein lies a major problem. The level

of coliform indicator bacteria in cold and moderate climate waters is related to the amount of fecal matter encountered by this water. In these waters, fecal coliforms maintain their biological characteristics, which are different from either transmissive stages of protozoans (e.g. *Cryptosporidium* and *Giardia*) or from fungal enteropathogens (e.g. microsporidian spores) evolutionarily designed to encounter harsh environmental conditions. Many studies have shown the inadequacy of standard fecal coliforms when used as indicators, predictors or markers for biological contaminants in drinking, recreational, seafood-harvesting or waste waters [7–10]. Unfortunately, a vast range of human protozoan enteropathogens have a zoonotic reservoir, meaning that aquatic animals can sustain their source [11,12] and if the microbiological load for these animals is not related to the fecal coliform level, standard drinking water procedures (e.g. flocculation, sedimentation, filtration and disinfection) might not be adequately and promptly adjusted to produce safe finished water.

Waterborne protozoan and fungal enteropathogens

Cryptosporidium parvum, *Giardia lamblia* and human-irulent microsporidia, such as *Enterocytozoon bienersi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem* and *Encephalitozoon cuniculi*, are human anthroponotic pathogens that inflict considerable morbidity (diarrheal disease) on healthy people, and some (e.g. *Cryptosporidium*) can cause mortality in immunosuppressed individuals [13–15]. *Cryptosporidium* and *Giardia* are frequently transmitted via water [13,14], and numerous reports indicate involvement of water in the epidemiology of microsporidian spores [16]. The transmissive stages (i.e. oocysts, cysts and spores) are environmentally robust and therefore ubiquitous in aquatic habitats [7,13,17]. These pathogens are category B biodefense agents on the National Institutes of Health (NIH) list, and microsporidian spores are on the Contaminant Candidate List of the US Environmental Protection Agency (EPA) because spore identification, removal or inactivation in drinking water is technically challenging [18]. No specific legislation exists for the routine monitoring of drinking water sources or recreational waters for these pathogens, and considerable evidence demonstrates their direct zoonotic association with birds, including waterfowl species (Table 1).

Corresponding author: Graczyk, T.K. (tgraczyk@jhsph.edu).

Table 1. *Cryptosporidium* oocysts, *Giardia* cysts and spores of human-virulent microsporidian species reported from birds

Pathogen species	Avian species	Comments	Refs
<i>Cryptosporidium</i> sp.	<i>Larus</i> spp.	5% of fecal and 22% of cloacal lavage samples positive; 64% and 83% oocysts, respectively potentially viable.	[1]
<i>C. parvum</i>	<i>Branta canadensis</i>	Migratory geese; oocysts infectious to neonatal Balb/c mice; oocyst concentration range 6.7×10^2 – 6.9×10^3 g ⁻¹ ; mean 3.7×10^3 g ⁻¹ .	[11]
<i>Cryptosporidium</i> sp.	<i>Anas discour</i> , <i>A. cerca carolinensis</i> , <i>A. platyrhynchos</i> , <i>A. americana</i> , <i>Lophodytes cucullanus</i> , <i>Mergus merganser</i>	Migratory ducks; 49% birds positive; PCR did not confirm <i>C. parvum</i> ; oocyst concentration range 0– 2.0×10^3 g ⁻¹ ; mean 48×10^3 g ⁻¹ .	[28]
<i>C. parvum</i> , <i>C. hominis</i>	<i>B. Canadensis</i>	Residential and migratory geese, 2.4% samples positive, novel avian genotypes identified; oocysts acquired from local unhygienic sites.	[21]
<i>Cryptosporidium</i> sp.	<i>B. Canadensis</i>	Residential and migratory geese, 81% and 90% of fecal samples from collection sites positive.	[29]
<i>Giardia</i> sp.	<i>B. Canadensis</i>	Migratory geese; cyst concentration range 7.5×10^2 – 7.9×10^3 g ⁻¹ ; mean 4.1×10^3 g ⁻¹ .	[11]
<i>Giardia</i> sp.	<i>A. discour</i> , <i>A. platyrhynchos</i> , <i>A. americana</i> , <i>A. acuta</i> , <i>M. merganser</i>	Migratory ducks; cyst concentration range 0– 2.9×10^4 g ⁻¹ ; mean 4.4×10^3 g ⁻¹ .	[28]
<i>Encephalitozoon hellem</i>	<i>A. platyrhynchos</i> , <i>Anser anser</i> , <i>C. olor</i> , <i>C. atratus</i> , <i>C. malanocoryphus</i> , <i>Coccoroba coscoroba</i> , <i>Balearica pavonina</i>	Free-ranging and captive birds; 8.6% birds positive; spore concentration range 2.0×10^3 – 5.1×10^5 g ⁻¹ ; mean 3.6×10^5 g ⁻¹ ; spores potentially viable.	[12]
	<i>Columba livia</i>	Urban feral pigeons; 0.8% pigeons positive for <i>E. hellem</i> ; 4.8% pigeons <i>E. hellem</i> – <i>E. bienewisi</i> co-infected; 0.8% pigeons <i>E. hellem</i> – <i>E. intestinalis</i> co-infected.	[24]
<i>E. intestinalis</i>	<i>A. a. domestica</i>	A livestock goose; spore concentration, 4.0×10^5 g ⁻¹ ; spores potentially viable.	[12]
	<i>C. livia</i>	Urban feral pigeons; 4% pigeons positive for <i>E. intestinalis</i> ; 0.8% pigeons <i>E. intestinalis</i> – <i>E. bienewisi</i> co-infected.	[24]
<i>Enterocytozoon bienewisi</i>	<i>C. livia</i>	Urban feral pigeons; 9.7% pigeons positive.	[24]
	<i>C. livia</i>	Seven novel genotypes described; all similar to pig, raccoon and human genotypes.	[25]
	17 species of caged-pet birds, <i>C. livia</i>	Captive birds and urban feral pigeons; 28.9% birds positive; genotype reported previously from HIV/AIDS patients.	[26]
	<i>C. livia</i>	Spore concentration range 3.5×10^3 – 3.7×10^3 g ⁻¹ ; mean 3.6×10^3 g ⁻¹ ; 85% of spores potentially viable.	[27]

Waterfowl as vectors of etiological agents of human disease

The vectorial capacity of waterfowl for delivering human pathogens to surface water is influenced by various host-related and pathogen-specific factors. Host-related factors result in either the establishment of infection in birds (i.e. the reservoir host) or the lack of infection (i.e. mechanical transfer, passage or carrying of the pathogen). These mechanisms are not fully understood. *Cryptosporidium parvum* and *C. hominis* can be mechanically passed to water by birds in feces, and this has been documented experimentally [19,20] and in the field [11,21]. Frequently, migratory herbivorous birds follow cattle (reservoir host of *C. parvum*) and take advantage of undigested plant material in cattle feces [11]. However, residential wild geese can acquire *C. hominis* oocysts from local garbage and other unhygienic places [21]. Because mankind is the predominant source of *C. hominis*, it is reasonable to assume that finding this species in goose feces [21] indicates a mechanical transfer. By contrast, *Giardia* has an extensive zoonotic reservoir [13]. The cysts of assemblages virulent to humans are common in water [22] and can be acquired by birds from this environment. A single avian isolate of *Giardia* was virulent to mammals [23], indicating that birds can serve as reservoir hosts in addition to being mechanical vectors (Table 1). Human-virulent microsporidian species are the least-studied group from the environmental health sciences standpoint.

E. hellem can readily infect birds and has been reported from seven waterfowl species, and *E. intestinalis* has been reported from one species [12]. Alarming, increasing numbers of worldwide reports show the association of feral urban pigeons with human-virulent microsporidian species (Table 1), particularly *E. bienewisi* [24–27], including *E. bienewisi* genotypes typically identified in HIV/AIDS patients [25,26].

Vectorial capacity of birds

The prevalence of birds shedding human pathogens in feces and the associated pathogen intensity varies (Table 1). In one study, concentrations of infectious *C. parvum* oocysts (as per mouse bio-assay) and *Giardia* sp. cysts in fecal droppings of migratory Canada geese were 3.7×10^3 g⁻¹ and 4.10×10^3 g⁻¹, respectively [11]. The concentration of *Cryptosporidium* sp. oocysts and *Giardia* sp. cysts in migratory ducks was 4.8×10^2 g⁻¹ and 4.4×10^4 g⁻¹, respectively [28]. The prevalence of bird fecal droppings positive for *Cryptosporidium* sp. oocysts can be as high as 90% [29]. The concentration of *E. hellem* spores varies from 2.0×10^3 g⁻¹ to 5.0×10^5 g⁻¹ in feces, with a mean value for various waterfowl species of 3.6×10^5 g⁻¹ [12]. The overall concentration of *E. bienewisi* spores was 3.6×10^3 g⁻¹ in excrement of urban feral pigeons [27]. Such high concentrations of pathogen-transmissible stages shed in bird feces indicate indigenous infection rather than a mere mechanical carriage of pathogens acquired from the

Opinion

environment. What are the relevant implications for the quality of surface waters used for drinking purposes or recreation? The fecal coliform input by birds to surface water has recently been characterized by a field databased prediction model for the microbial-quality impact inflicted by waterfowl visitation [2]. For quantitative assessment, the model considers the number of fecal pellets (n) deposited on a 100 m long and 1 m wide shore-line by a single visitation of an average flock of ducks, geese or gulls. The n value reached 1700 [2]. Thus, considering an average waterfowl fecal pellet weight of 17.2 g [11], with 8.6% of birds shedding human pathogens [12], and the concentration of *C. parvum*, *Giardia* sp. and *E. hellem* shed in bird feces [11,12], a single visitation of an average size waterfowl flock can introduce into the water approximately 9.3×10^6 infectious *C. parvum* oocysts, 1.0×10^7 *Giardia* sp. cysts and 9.0×10^8 *E. hellem* spores [12]. However, is this a large quantity? How much is enough to infect humans and seriously affect water quality? *Cryptosporidium parvum* isolates differ in their virulence to humans [30,31]. Volunteer challenge trials that used healthy *C. parvum*-seronegative individuals showed that the ID₅₀ (infectious dose 50%, i.e. the number of pathogens that can cause infection in 50% of exposed population) or minimal infectious dose for the three main *C. parvum* isolates [i.e. TAMU (Texas A & M University), IOWA and UCP (Ungar *Cryptosporidium parvum*)] varied from 9 to 18, from 87 to 190 and from 1042 to 2980 oocysts, respectively [30,31]. Giardiasis in immunocompetent people can be caused by 10 cysts [32], and because microsporidia are emerging pathogens, their ID₅₀ are unknown and await elucidation by volunteer experimental challenge studies. However, based on animal data the infectious dose is thought to be low [15]. In water, the oocysts, cysts or spores retain their infectivity for a prolonged period – from two months for *Giardia* [32] and several months for microsporidian spores [17] to a year for *Cryptosporidium* [33], which facilitates transmission via recreational contact. In addition, the small sizes and resistance of oocysts and spores to standard water chlorination facilitates transmission via drinking water [7,17].

The future of water safety assessment

Advances in molecular technology together with a competitive market made many molecular epidemiology techniques affordable and routinely used for identification of waterborne enteropathogens. Species-specific identification of pathogens is essential for water monitoring; however, currently used immunofluorescent antibodies (IFA) for identification of waterborne *Cryptosporidium* produce a positive reaction with oocyst species not virulent to people [34]. Of equal importance to species-specific identification is the assessment of pathogen viability or infectivity. For example, a standard protocol for reporting of *Cryptosporidium* in drinking water in the USA requires species identification, oocyst enumeration and assessment of their viability (United States Environmental Protection Agency; <http://www.epa.gov/nerlcwww/1623de05.pdf>). Of all viability and infectivity assays on the market [35], the suckling BALB/c mouse bio-assay provides the ultimate answer to oocyst infectivity issues; in fact, this

assay first showed the infectivity of *C. parvum* oocysts in waterfowl fecal droppings [11]. However, the high inoculum size and the false-negative outcome for *C. hominis*, which does not readily infect animals [36], make this method unsuitable for water testing. Therefore, considerable efforts by regulatory agencies concerned with water safety are focused on the development of method(s) that will enable quantitative, species-specific and simultaneous detection of multiple pathogens with assessment of their viability [37]. One of these techniques is fluorescent *in situ* hybridization (FISH) in combination with IFA, which enables multiplexed quantitative species-specific identification of *C. parvum*, *C. hominis*, *G. lamblia* [38] and all four human-virulent microsporidian parasites [39], together with viability assessment of oocysts, cysts and spores [12,16,38,39].

Concluding remarks and future directions

The aquatic environment is a shared resource, required year-round by all species of waterfowl and by humans. Management of drinking and recreational water resources should implement effective protection measures from aquatic birds, and should be ecologically friendly and ethically acceptable. The spectrum of protection measures depends on the geographical and ecological characteristics of the relevant water reservoirs.

The environmental and ecological interactions of waterfowl and humans will inevitably continue to have negative public health consequences for drinking water resources. This highlights the urgent need for better water-quality indicators, or alternatively, for testing the source waters for *Cryptosporidium* and human-virulent microsporidia. European countries are evaluating the environmentally resistant spore-forming bacteria *Clostridium perfringens* as a water-quality indicator (<http://www.carlow.ie/services/environment/reports/CryptoOutbreakCarlow2005.pdf>). Drinking water with acceptable fecal coliform levels has caused outbreaks of giardiasis, and the cysts have been traced to beavers living in the source water reservoir [40]. This resulted in a reduction of the *Giardia* threat to drinking water by leading to the introduction of properly performed filtration and chlorination, and established an understanding that improvement of water quality by lowering fecal coliform counts is not a sound solution for waterborne protozoan pathogens. Source tracking of fecal coliforms does not offer a satisfactory solution to water safety assessment; however, it can aid in the identification of fecal pollution sources and contribute to watershed protection [5,6].

Considerable evidence indicates that migratory birds can contribute to the global spread of infectious agents in a spatial manner analogous to humans traveling on aircraft [41]. Waterfowl species: (i) usually occur in large flocks, (ii) can migrate long distances, (iii) frequently graze and defecate in water and (iv) are protected by environmental laws in many regions where they have unlimited access to surface waters used for drinking water production [11,12].

Waterfowl are part of our natural resource heritage and are necessary for the proper functioning of aquatic ecosystems. In the areas where their presence is environmentally justifiable, these birds should be supported and protected.

However, to protect the public health, managers of surface waters used for drinking water abstraction and recreation that are vulnerable to microbiological contamination from birds should apply effective protection measures. The demand for microbiologically safe water grows exponentially owing to the rise in human populations. As postulated already [42], revision of current legislation is required in tandem with the development of new control measures, hopefully with the approval of animal rights activists.

Advances in molecular technology have prompted multiple diagnostic discoveries related to human infectious agents. The genetic diversity of bird-specific *Cryptosporidium* is substantial [43], and many molecular laboratories do not always have sufficient epidemiological and environmental science expertise to accurately assess the epidemiological relevance and importance of their results. For example, multiple novel *Cryptosporidium* 'goose' and 'duck' genotypes [21,44,45] have not been seen in humans, and the virulence in humans of *Cryptosporidium* sp. oocysts found in 90% of goose and 49% of duck droppings [28,29] is unknown. To maintain the public health, it is essential that accurately interpreted information is provided to regulatory agencies, drinking and recreational water industries, and the public.

The high quality of drinking water produced from potable sources and microbiologically safe surface recreational waters is the outcome of a partnership between engineering, environmental health and epidemiological sciences, reinforced by regulatory agencies. The mounting evidence provided by academia on the negative impact inflicted by birds residing at our usable water resources needs to be appropriately addressed by regulatory agencies. Current technology allows for multiplexed species-specific identification, enumeration, viability assessment and source tracking of human protozoan pathogens in water. Such technology could be implemented into water-monitoring programs chiefly to benefit public health and, additionally, to add to further assessment of the impact of wildlife on water resources.

Acknowledgements

We apologize for not citing all original articles owing to space constraints. The studies on waterfowl and human pathogens were supported by the Fulbright Senior Specialist Fellowship to T.K.G. (2225), Johns Hopkins National Institute for Environmental Health Sciences Center in Urban Environmental Health (P30 ES03819), Johns Hopkins Faculty Research Innovation Fund, Johns Hopkins Center for a Livable Future, and U.S. Environmental Protection Agency Science to Achieve Results (STAR) Program (RD83300201). The views expressed herein have not been subjected to the U.S. Environmental Protection Agency review and therefore do not necessarily reflect the views of the agency, and no official endorsement should be inferred.

References

- Smith, H.V. *et al.* (1993) Occurrence of oocysts of *Cryptosporidium* sp. in *Larus* spp. gulls. *Epidemiol. Infect.* 110, 135–143
- Kirschner, A.K. *et al.* (2004) Integral strategy for evaluation of fecal coliform indicator performance in bird-influenced saline inland waters. *Appl. Environ. Microbiol.* 70, 7396–7403
- Standridge, J.H. *et al.* (1979) Effect of waterfowl (*Anas platyrhynchos*) on indicator bacteria populations in a recreational lake Madison, Wisconsin. *Appl. Environ. Microbiol.* 38, 547–550
- Hussong, D. *et al.* (1979) Microbial impact of Canada geese (*Branta canadensis*) and Whistling swans (*Cygnus columbianus columbianus*) on aquatic ecosystems. *Appl. Environ. Microbiol.* 37, 14–20
- Middleton, J.H. and Ambrose, A. (2005) Enumeration and antibiotic resistance patterns of fecal indicator organisms isolated from migratory Canada geese (*Branta canadensis*). *J. Wildl. Dis.* 41, 334–341
- Duran, M. *et al.* (2006) Microbial source tracking using host specific FAME profiles of fecal coliforms. *Water Res.* 40, 67–74
- Smith, H.V. and Rose, J.B. (1998) Waterborne cryptosporidiosis: current status. *Parasitol. Today* 14, 14–22
- Schwab, K.J. (2007) Are existing bacterial indicators adequate for determining recreational water illness in waters impacted by nonpoint pollution? *Epidemiology* 18, 21–22
- Graczyk, T.K. and Schwab, K.J. (2000) Foodborne infections vectored by molluscan shellfish. *Curr. Gastroenterol. Rep.* 2, 305–309
- Rimhanen-Finne, R. *et al.* (2004) Comparative analysis of *Cryptosporidium* and *Giardia* and indicator bacteria during sewage sludge hygienization in various composting processes. *Lett. Appl. Microbiol.* 38, 301–305
- Graczyk, T.K. *et al.* (1998) *Giardia* sp. and infectious *Cryptosporidium parvum* oocysts in the feces of migratory Canada geese (*Branta canadensis*). *Appl. Environ. Microbiol.* 64, 2736–2738
- Slodkiewicz-Kowalska, A. *et al.* (2006) Microsporidia species known to infect humans are present in aquatic birds; implications for transmission via water? *Appl. Environ. Microbiol.* 72, 4540–4544
- Wolfe, M.S. (1992) Giardiasis. *Clin. Microbiol. Rev.* 5, 93–100
- Graczyk, T.K. *et al.* (1997) Zoonotic potential of *Cryptosporidium parvum*: implications for waterborne cryptosporidiosis. *Parasitol. Today* 13, 348–351
- Weber, R. and Bryan, R.T. (1994) Microsporidial infections in immunodeficient and immunocompetent patients. *Clin. Infect. Dis.* 19, 517–521
- Graczyk, T.K. *et al.* (2004) Human waterborne parasites in zebra mussels (*Dreissena polymorpha*) from the Shannon River drainage, Ireland. *Parasitol. Res.* 93, 385–391
- Kucerova-Pospisilova, Z. *et al.* (1999) Environmental resistance of *Encephalitozoon* spores. *J. Eukaryot. Microbiol.* 46, 11S–13S
- U.S. Environmental Protection Agency (EPA) (1998) Announcement of the drinking water contaminant candidate list: notice. *Fed. Regist.* 63, 10272–10287
- Graczyk, T.K. *et al.* (1996) Viability and infectivity of *Cryptosporidium parvum* oocysts are retained upon intestinal passage through a refractory avian host. *Appl. Environ. Microbiol.* 62, 3234–3237
- Graczyk, T.K. *et al.* (1997) Infectivity of *Cryptosporidium parvum* oocysts is retained upon intestinal passage through a migratory waterfowl species (Canada goose, *Branta canadensis*). *Trop. Med. Int. Health.* 2, 341–347
- Zhou, L. *et al.* (2004) Host-adapted *Cryptosporidium* spp. in Canada geese (*Branta canadensis*). *Appl. Environ. Microbiol.* 70, 4211–4215
- Graczyk, T.K. *et al.* (1999) *Giardia duodenalis* of genotype A recovered from clams in the Chesapeake Bay subestuary, Rhode River. *Am. J. Trop. Med. Hyg.* 61, 526–529
- Upercroft, J.A. *et al.* (1998) Virulent avian *Giardia duodenalis* pathogenic for mice. *Parasitol. Today* 14, 281–284
- Haro, M. *et al.* (2005) First detection and genotyping of human-associated microsporidia in pigeons form urban parks. *Appl. Environ. Microbiol.* 71, 3153–3157
- Haro, M. *et al.* (2006) Detection and genotyping of *Enterocytozoon bieneusi* in pigeons. *J. Eukaryot. Microbiol.* 53, S58–S60
- Lobo, M.L. *et al.* (2006) Identification of potentially human-pathogenic *Enterocytozoon bieneusi* genotypes in various birds. *Appl. Environ. Microbiol.* 72, 7380–7382
- Graczyk, T.K. *et al.* (2007) Urban feral pigeons (*Columba livia*) as a source for air-and-waterborne contamination with *Enterocytozoon bieneusi* spores. *Appl. Environ. Microbiol.* 73, 4357–4358
- Kuhn, R.C. *et al.* (2002) Occurrence of *Cryptosporidium* and *Giardia* in wild ducks along the Rio Grande river valley in Southern New Mexico. *Appl. Environ. Microbiol.* 68, 161–165
- Kassa, H. *et al.* (2004) Cryptosporidiosis: a brief literature review and update regarding *Cryptosporidium* in feces of Canada geese (*Branta canadensis*). *J. Environ. Health.* 66, 34–40

- 30 Okhuysen, P.C. *et al.* (1999) Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. *J. Infect. Dis.* 180, 1275–1281
- 31 Messner, M.J. *et al.* (2001) Risk assessment for *Cryptosporidium*: a hierarchical Bayesian analysis of human dose response data. *Water Res.* 35, 3934–3940
- 32 Rose, J.B. *et al.* (1991) Risk assessment and control of waterborne giardiasis. *Am. J. Public Health* 81, 709–713
- 33 Tamburrini, A. and Pozio, E. (1999) Long-term survival of *Cryptosporidium parvum* oocysts in seawater and in experimentally infected mussels (*Mytilus galloprovincialis*). *Int. J. Parasitol.* 29, 711–715
- 34 Graczyk, T.K. *et al.* (1996) Evaluation of commercial enzyme immunoassay (EIA) and immunofluorescent antibody (IFA) tests kits for detection of *Cryptosporidium* oocysts other than *Cryptosporidium parvum*. *Am. J. Trop. Med. Hyg.* 54, 274–279
- 35 Rochelle, P.A. *et al.* (2002) Comparison of *in vitro* cell culture and a mouse assay for measuring infectivity of *Cryptosporidium parvum*. *Appl. Environ. Microbiol.* 68, 3809–3817
- 36 Morgan-Ryan, U.M. *et al.* (2002) *Cryptosporidium hominis* n. sp. (Apicomplexa: Cryptosporidiidae) from *Homo sapiens*. *J. Eukaryot. Microbiol.* 49, 433–440
- 37 Jiang, J. and Xiao, L. (2003) An evaluation of molecular diagnostic tools for the detection and differentiation of human-pathogenic *Cryptosporidium* spp. *J. Eukaryot. Microbiol.* 50, 542–547
- 38 Graczyk, T.K. *et al.* (2007) Human enteropathogen load in activated sewage sludge and corresponding sewage sludge-end products. *Appl. Environ. Microbiol.* 73, 2013–2015
- 39 Graczyk, T.K. *et al.* (2007) Retrospective species identification of microsporidian spores in diarrhetic fecal samples from HIV/AIDS patients by multiplexed fluorescent *in situ* hybridization (FISH). *J. Clin. Microbiol.* 45, 1255–1260
- 40 Isaac-Renton, J.L. (1993) Characterization of *G. duodenalis* isolates from a waterborne outbreak. *J. Infect. Dis.* 167, 431–440
- 41 Reed, K.D. *et al.* (2003) Bird, migration and emerging zoonoses: West Nile virus, Lyme disease, influenza A, and enteropathogens. *Clin. Med. Res.* 1, 5–12
- 42 Dieter, R.A. *et al.* (2001) Zoonotic disease: health aspects of Canada geese. *Int. J. Circumpolar Health.* 60, 676–684
- 43 Ng, J. *et al.* (2006) Identification of novel *Cryptosporidium* genotypes from avian hosts. *Appl. Environ. Microbiol.* 72, 7548–7553
- 44 Jellison, K.L. *et al.* (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
- 45 Jellison, K.L. *et al.* (2007) Phylogenetic analysis implicates birds as a source of *Cryptosporidium* sp. Oocysts in agricultural watersheds. *Environ. Sci. Technol.* 41, 3620–3625