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Review

Global occurrence of *Cryptosporidium* and *Giardia* in shellfish: Should Canada take a closer look?

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ABSTRACT

The zoonotic protozoan parasites *Cryptosporidium parvum*, and *Giardia duodenalis* can colonize the gastrointestinal tracts of many farmed, domestic and wild animals, and are capable of causing disease in humans. Due to their high prevalence in dairy and beef cattle, farms are considered to be potential sources of transmission of the parasites to surrounding bodies of water. Additionally, human sewage overflow events can also contaminate nearby water sources. Both protozoa have been detected in various species of shellfish, but a lack of consistency in methods for detection and viability assessments make it difficult to compare data and extrapolate results. Nonetheless, the detection of these parasites in shellfish destined for human consumption is necessary due to the public health risk that these parasites pose. There is currently no legislation requiring public health officials to test for these parasites in potentially contaminated shellfish or water, despite the increasing evidence suggesting the need to implement parasitological analyses. Furthermore, standard depuration procedures are ineffective at inactivating the parasites, and even supplementation with ultraviolet radiation requires extensive periods of depuration to effectively inactivate all (oo)cysts from bivalves. Although there have been no official reports of human gastroenteritis acquired from consuming shellfish contaminated with *Cryptosporidium* sp. and/or *G. duodenalis*, studies examining the efficacy of current monitoring and depuration standards in Canada are needed in the event that they are inadequate for the removal of potentially harmful protozoan parasites.

Contents

1. Introduction

The protozoan parasites *Cryptosporidium* and *Giardia* are well known causative agents of gastrointestinal disease and are commonly identified in humans and animals, including livestock (Heitman et al.,

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2002; Xiao et al., 1999). Livestock, particularly cattle, harbour zoonotic *Cryptosporidium parvum* and *Giardia duodenalis* (assemblages A and B) and have potential for serious public health consequences (Fayer, Speer, & Dubey, 1997). Cryptosporidiosis cases in humans caused by *C. parvum* infections are far more prevalent in Europe, whereas the non-zoonotic *C. hominis* is the predominant health concern in North and South America, and Africa (Bushen et al., 2007; Fayer et al., 2003; Guyot et al., 2001; McLauchlin, Amar, Pedraza-Diaz, & Nichols, 2000; Yagita et al., 2001). *Giardia duodenalis* is a cosmopolitan species and is the most frequent intestinal parasite in developing countries, with over 200 million people infected globally and over 500,000 new cases annually (Ivanov, 2010; WHO, 1996).

Cryptosporidium and *Giardia* present public health challenges due to their low infective dose, ease of dispersal and persistence in the environment, difficulty to reliably isolate and detect, small (oo)cyst size that may bypass common water treatments and relative resistance to disinfection (Erickson & Ortega, 2006; Olson, Goh, Phillips, Guselle, & McAllister, 1999). Both parasites may be transmitted by direct fecal–oral route (and thus by direct contact with infected animals or humans) or by ingestion of food or water contaminated with (oo)cysts (Dixon et al., 2011).

Agricultural practices including manure storage and manure application to arable land, discharge of contaminated water, pasturing of livestock near water sources and disposal of fecally contaminated waste from abattoirs and wastewater treatment facilities may result in contamination of the environment with large numbers of (oo)cysts (Schijven, Bradford, & Yang, 2004; Slifko, Smith, & Rose, 2000). With (oo)cysts circulating in environmental water supplies, and waterborne outbreaks documented in developed countries throughout the world, attention has been drawn to transmission potential of parasites in contaminated shellfish to consumers. This review discusses the occurrence of *Cryptosporidium* sp. and *Giardia duodenalis* in Canadian cattle, wildlife, and water supplies as well as the global potential for shellfish contamination by these parasites. Through improved detection methods, studies continue to confirm the presence of these parasites in livestock and contaminated water sources in Canada. Although studies examining Canadian shellfish for these zoonotic protozoa are limited, literature available on bioaccumulation and retention of (oo)cysts in shellfish highlights the need to examine the efficacy of current sanitation and depuration protocols in Canada to prevent the public from acquiring cryptosporidiosis and/or giardiasis from the consumption of contaminated shellfish.

2. Canadian cattle and wildlife as sources for zoonotic *Cryptosporidium* sp. and *Giardia duodenalis*

Cryptosporidium sp. and *Giardia duodenalis* can infect many farmed animals, including sheep, cattle, goats, swine, and horses (Budu-Amoako et al., 2012a; Farzan et al., 2011; Olson et al., 1999). Currently there are 24 species of *Cryptosporidium* supported by genetic studies, but only *C. parvum*, *C. hominis*, and *C. meleagridis* are known to be pathogenic to humans. *C. parvum* poses the greatest transmission risk from farm animals to surrounding environments. Of the seven *G. duodenalis* assemblages (A–G), only A and B are zoonotic, while assemblage E is livestock specific and non-zoonotic (O'Handley & Olson, 2006). During peak shedding, infected animals can excrete ~10^7 (oo)cysts/g of feces for several days, with daily output exceeding 10^9 (oo)cysts (O'Handley & Olson, 2006; Xiao, Herd, & Bowman, 1994). Higher infection rates and (oo)cyst shedding occur in younger animals which make them the major source of these parasites (Ralston, McAllister, & Olson, 2003; Xiao & Herd, 1994; Xiao et al., 1999). Peak shedding of *G. duodenalis* occurs in calves ~5 weeks old (Xiao et al., 1994), while for *C. parvum*, peak shedding occurs in calves 1–2 weeks old (Ralston et al., 2003).

Cryptosporidium sp. and *Giardia duodenalis* prevalence varies widely in cattle across Canada. Comparisons between studies and even sites within studies are difficult to make as prevalence rates vary as a result of climate, season, age of calf/cattle, farm management practices, and sensitivity of diagnostic tests for parasite isolation and enumeration (Dixon, 2008). For instance, it has been reported that *C. parvum* is less prevalent in beef calves than dairy calves in Canada (Mann, Sekia, Nayar, & Koschik, 1986; O'Handley & Olson, 2006), whereas other studies indicate the opposite (McAllister, Olson, Fletch, Wetzstein, & Entz, 2005). Parallel studies employing the same methodology and detection methods among beef and dairy cattle of Prince Edward Island (PEI) determined that *Cryptosporidium* was present in 80% of beef farms and 17% of the total fecal samples examined, whereas *Cryptosporidium* was only present in 55% of dairy farms, with 14% of total fecal samples examined positive for oocysts (Budu-Amoako et al., 2012b; Budu-Amoako, Greenwood, Dixon, Barkema, & McClure, 2012).

Additionally, earlier Canadian studies failed to distinguish zoonotic from host-specific species/assemblages, focusing only on microscopic examinations (Gow & Waldner, 2006; Heitman et al., 2002; Mann et al., 1986; McAllister et al., 2005; Olson et al., 1997; Olson, Thorlakson, Deselliers, Morck, & McAllister, 1997; Ruest, Faubert, & Couture, 1998; Trotz-Williams et al., 2005; 2008). The zoonotic risk and farm prevalence for zoonotic *Cryptosporidium* and/or *Giardia* therefore may be overestimated in many studies. For example, studies have detected *Giardia* in cattle with prevalence of 100% (O'Handley et al., 1999; Ralston et al., 2003), but genomic data later revealed that only 20% of these infections were likely caused by the zoonotic assemblage A (Ivanov, 2010; O'Handley, Olson, Fraser, Adams, & Thompson, 2000). With the advent of PCR for multilocus genotyping to confirm parasite identity, discrepancies between previous reports and the true prevalence of species/assemblages of public health concern in Canadian livestock can be clarified. Using a direct immunofluorescence assay (IFA) and microscopy, the on-farm prevalence for *Cryptosporidium* sp. was found to be ~64% for both dairy and beef cattle in Ontario, however molecular analysis revealed that 50% of IFA-positive dairy farm samples were *C. parvum* compared to 0% in beef farm samples (Dixon et al., 2011). In Alberta studies, the zoonotic *G. duodenalis* assemblage A is detected more frequently in cattle than assemblage B (Appelbee, Frederick, Heitman, & Olson, 2003; O'Handley et al., 2000), whereas assemblage B is more frequently detected in Ontario (Coklin, Farber, Parrington, & Dixon, 2007; Dixon et al., 2011). Overall prevalences of zoonotic *G. duodenalis* assemblages in Canadian cattle are very low in comparison to host-adapted assemblage E. Studies in PEI cattle determined that 88% of IFA-positive *Giardia* fecal samples that underwent PCR analyses were assemblage E, while the remaining 12% represented mixed infections of several assemblages (Uehlinger et al., 2011). Other studies on PEI showed similar results, as 89% and 90% of *Giardia* isolates from beef and dairy cattle, respectively, were genotyped as assemblage E (Budu-Amoako, Greenwood, Dixon, Barkema, Hurnik, et al., 2012b; Budu-Amoako, Greenwood, Dixon, Barkema, & McClure, 2012). Of the remaining IFA-positive *Giardia* samples, 4% and 7% of beef cattle isolates as well as 6% and 4% of dairy cattle isolates were assemblage A and assemblage B, respectively.

Several studies correlated increased numbers of (oo)cysts in surface water directly with increased densities of domestic livestock (Keeley & Faulkner, 2008; LeChevallier, Norton, & Lee, 1991; Ong, Moorehead, Ross, & Isaac-Renton, 1996). This environmental contamination may occur from agricultural runoff or through the application of fresh manure to fertilize agricultural lands (Dixon et al., 2008). Young ruminants are perceived as a source for waterborne *Cryptosporidium* and *Giardia* due to high prevalence of these parasites (O'Handley & Olson, 2006) and magnitude of (oo)cysts shed from infected animals (Fayer, Trout, Graczyk, & Lewis, 2000; Goh et al., 2004).

Genetic data is crucial in determining whether cattle are indeed a significant source of zoonotic *Cryptosporidium* oocysts and/or *Giardia* cysts found in contaminated waterways and human populations. For instance, a study in PEI examining a biased set of 658 human fecal samples found *Cryptosporidium* sp. in 22% of samples, of which *C. parvum* comprised 72% (Budu-Amoako et al., 2012). This study concluded that the presence