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Prevalence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in dairy cattle in Ontario, Canada

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Abstract

Giardia duodenalis and *Cryptosporidium* spp. are intestinal protozoan parasites that infect a wide range of host species, including humans. Molecular characterization of these parasites has demonstrated that a number of genotypes and species are common to both humans and animals, and that zoonotic transmission may occur. Numerous studies have reported a high prevalence of *G. duodenalis* and *Cryptosporidium* spp. in cattle, particularly calves, and these animals are frequently associated with zoonotic transmission. In the present study, a total of 143 faecal samples from adults, heifers and calves were collected from two dairy cattle farms in eastern Ontario, Canada. The prevalence and molecular characteristics of *G. duodenalis* and *Cryptosporidium* spp. in these animals were determined in order to investigate the potential for transmission between cattle and humans in this region. Following DNA extractions from faecal samples, nested-PCR protocols were used to amplify fragments of the 16S rRNA gene and the heat-shock protein 70 (HSP-70) gene for determining the prevalence of *G. duodenalis* and *Cryptosporidium* spp. infections, respectively. Genotypes of *G. duodenalis*, and species of *Cryptosporidium*, were determined by means of DNA sequencing of amplicons, and subsequent sequence alignment. Cattle on both farms showed a high prevalence of *G. duodenalis* (42.0%) and *Cryptosporidium* spp. (27.3%). *G. duodenalis* infections were more prevalent in calves and heifers than in adults, and *Cryptosporidium* spp. infections were only observed in calves and heifers. The zoonotic genotype, *G. duodenalis* Assemblage B was isolated from 24.5% of the cattle tested, while *G. duodenalis* Assemblage E was found in 17.5% of the cattle tested. The overall prevalence of the zoonotic species *Cryptosporidium parvum* in the animals tested was found to be 21.7%, while only 1.4% were infected with *C. bovis*. These findings suggest that there is a potential risk of zoonotic and/or zooanthroponotic transmission of *G. duodenalis* and *C. parvum* infections between cattle and humans in eastern Ontario, likely by means of contaminated water or food, or through direct faecal-oral transmission in the case of farmers and veterinary staff.

Keywords: *Giardia duodenalis*; *Cryptosporidium*; Cattle; Zoonotic risk; Genotyping

1. Introduction

Giardia duodenalis and *Cryptosporidium* spp. are protozoan parasites which infect a wide range of hosts, including humans, worldwide. *G. duodenalis* is the causative agent of giardiasis, a gastrointestinal disease

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causing diarrheal illness. Giardiasis is most widely recognized among travelers to developing countries (Ekdahl and Andersson, 2005), children in day-care centres (Keystone et al., 1984), and homosexual males (Keystone et al., 1980). There is also a significant incidence of giardiasis in developed countries. For example, in the United States an average of 22,000 cases of giardiasis were reported each year from 1998 to 2002 (Hlavsa et al., 2005). Transmission usually occurs through the direct faecal-oral route or through cyst contaminated water or food. *Cryptosporidium* spp. inhabit epithelial cells of the digestive organs and cause diarrheal illness, known as cryptosporidiosis, which may be very severe in immunocompromised humans, and cannot currently be treated effectively. As with giardiasis, transmission of cryptosporidiosis can occur via the direct faecal-oral route or ingestion of oocyst contaminated food or water. There has also been considerable interest in recent years in the potential for zoonotic transmission of *G. duodenalis* and *Cryptosporidium* spp., particularly with respect to cattle and other livestock.

A large number of prevalence studies have been done worldwide in recent years on *Giardia* and *Cryptosporidium* in livestock. The prevalence of both parasites has generally been reported to be high in dairy cattle, particularly calves, in which a 100% cumulative prevalence has been reported (Xiao and Herd, 1994; O’Handley et al., 1999; O’Handley, 2002). Studies in Canada have reported a prevalence in dairy calves of between 45.7% and 73% for *G. duodenalis*, and between 40.6% and 88.7% for *Cryptosporidium* spp. (Olson et al., 1997a; Ruest et al., 1998; O’Handley et al., 2000; Trotz-Williams et al., 2005). In the United States, *Cryptosporidium* spp. prevalences of between 7.5% and 49% have been reported in dairy calves (Garber et al., 1994; Nydam et al., 2005; Starkey et al., 2005). Trout et al. (2004, 2005) examined the prevalence and genotypes of *G. duodenalis* in pre- and post-weaned dairy calves from several states in the United States. These authors reported a widely varying prevalence of 9–93%, with an average of 40% in pre-weaned calves and an overall prevalence of 52% in post-weaned calves.

Several studies have examined *Cryptosporidium* and *Giardia* infections in older dairy cattle, and have reported a generally lower prevalence than in calves (Fayer et al., 2000a, 2006, 2007; Santín et al., 2004). For example, a prevalence study of *Cryptosporidium* and *Giardia* infections was done in post-weaned and adult cattle in Maryland, United States (Fayer et al., 2000a). Out of a total of 24 cows analyzed at a research dairy farm, 12.5% were positive for *C. andersoni*, while no *Giardia* cysts were found. In the same study, at a commercial dairy operation, *C. parvum* and *G. duodenalis* were only found in 2 out of the 19 cows analyzed. Conversely, Trout et al. (2006) reported a relatively high and varied prevalence of *Giardia* in 1–2-year-old dairy cattle. Uehlinger et al. (2006) also reported a high prevalence of *G. duodenalis* in adult dairy cattle in a veterinary college teaching herd, along with a complete absence of *Cryptosporidium* spp.

Molecular characterization of *G. duodenalis* isolates have revealed seven major genotypes (Assemblages) with differing host specificities (Monis et al., 1999, 2003; Thompson et al., 2000; Thompson, 2004; Thompson and Monis, 2004). Assemblages A and B are the zoonotic *G. duodenalis* genotypes, and represent the genotypes with the widest host range including humans, cattle and many other mammals. Humans are in fact only susceptible to infection with these two genotypes (Caccio et al., 2005). All other Assemblages are host specific, and include: Assemblages C and D in dogs, Assemblage E in hoofed livestock, Assemblage F in cats, and Assemblage G in rats (Monis et al., 2003; Thompson, 2004; Thompson and Monis, 2004).

Cattle may be infected with either the zoonotic *G. duodenalis* Assemblage A or the host adapted, non-zoonotic Assemblage E. *G. duodenalis* Assemblage B has also been detected in a wide range of mammalian hosts, and has recently been reported in cattle (van Keulen et al., 2002; Lalle et al., 2005; Mendonça et al., 2007) and sheep (Castro-Hermida et al., 2007). Numerous studies examining the genotypes of *G. duodenalis* in cattle have reported a higher prevalence of Assemblage E than Assemblage A (O’Handley et al., 2000; van Keulen et al., 2002; Appelbee et al., 2003; Trout et al., 2004, 2005, 2006, 2007; O’Handley and Olson, 2006). Olson et al. (2004) concluded that this likely limits the role of cattle as reservoirs for human infection. However, in four multi-state prevalence studies for *G. duodenalis* in the eastern United States, pre-weaned calves, post-weaned calves, 1–2-year-old heifers, and adult dairy cows were all reported to harbor varying levels of the zoonotic Assemblage A (Trout et al., 2004, 2005, 2006, 2007). A similar prevalence of both Assemblages A and E was reported by Uehlinger et al. (2006) in adult dairy cattle in a veterinary college teaching herd, and the authors concluded that there was a potential zoonotic risk of infection to students and staff. Recently, O’Handley and Olson (2006) indicated that the presence of Assemblage A in ruminants may, in fact, be suggestive of a human source of infection.

Molecular studies have provided considerable evidence of genetic heterogeneity among isolates of