

Molecular epidemiology of *Giardia duodenalis* and *Cryptosporidium* spp on swine farms in Ontario, Canada

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Abstract

A subset of swine farms in Ontario, Canada have been monitored for *Cryptosporidium* and *Giardia*. Fecal samples were collected from different stages of production as well as from manure pits. *G. duodenalis* cysts and *Cryptosporidium* spp. oocysts were detected in the manure samples using immunofluorescence microscopy. A nested PCR and sequencing method was performed to determine the genotypes. A mixed multivariable method was used to compare the prevalence of *Cryptosporidium* and *Giardia* in samples from different sources. *Cryptosporidium* oocysts and *Giardia* cysts were recovered from 51.0% and 44.3% of samples, respectively. However, using PCR, 66.4% of fecal samples were positive for *Giardia* and 55.7% for *Cryptosporidium*. *Cryptosporidium* was more likely detected in manure pits and weaners compared to finisher pigs but it was less frequent in sows than in finishers ($P < 0.05$). Prevalence of *Giardia* was less frequent among sows and weaners compared to finisher pigs ($P < 0.05$). In total, 92% of the *Giardia* isolates were Assemblage B and 8% were Assemblage E. The most prevalent *Cryptosporidium* genotypes were *C. parvum* (55%) and pig genotype II (38%). Only one (2%) of the *Cryptosporidium* spp. isolates was determined to be *C. suis*. These findings indicate that the occurrence of zoonotic *G. duodenalis* and *Cryptosporidium* are very high on swine farms in southern Ontario, and that there is a potential for transmission between swine and humans by means of cyst and oocyst contaminated water or foods.

Introduction

Gastrointestinal illnesses continue to be the important public health issue globally (Kosek et al. 2003) with a significant economic impact (Mead et al, 1999). In Canada, gastrointestinal diseases associated with *Giardia* and *Cryptosporidium* has been reported as one of the top three enteric illnesses in Canada (Demczuk et al., 2005). *Giardia duodenalis* (also is known as *Giardia duodenalis* and *Giardia lamblia*) and *Cryptosporidium* spp. are ubiquitous parasites and in addition to human can infect a wide range of mammalian species causing asymptomatic to severe intestinal infections. Although human, livestock, and wildlife have been shown as potential source of *Giardia* and *Cryptosporidium* in the environment (Heitman et al, 2002), a little is known about attribution of each source to the presence of those parasites in environment. The infection of *Cryptosporidium parvum* in human in developed countries is mostly attributed to animals (Xiao, 2010). The infective *Cryptosporidium* oocysts and *Giardia* cysts are shed by infected animals and can survive for a long period in moist and cool environments (Olson et al, 2004). It is possible that those zoonotic agents get into ground water and contaminate the environment if spreading on land as fertilizer.

G. duodenalis and *Cryptosporidium* spp. infections in swine have generally been reported as being asymptomatic (Sanford, 1987), thereby the apparently healthy pigs may shed infective oocysts into environment. Exposure to infective oocysts/cysts through the contaminated water, food and produces is an important mode of infection in human. The aim of this paper is to describe the prevalence, as well as the genotypes and species of *G. duodenalis* and *Cryptosporidium* spp among pigs in different stage of production and in stored manure on a subset of Ontario swine farms in Canada.

Material and Methods

Ten swine farms were visited three times between September 2005 and May 2006. In each visit, fecal samples were collected from the stored manure pit and fresh samples obtained from finishers, sows, and weaners. In total, 122 fecal samples (31 manure pit, 43 finishers, 24 sows, and 24 weaners) were collected over the entire period of the study.

G. duodenalis cysts and *Cryptosporidium* spp. oocysts were detected in the manure samples using immunofluorescence microscopy. Also a nested PCR was used to determine the presence of *Giardia* and *Cryptosporidium* in feces. PCR products were sequenced to determine species and genotypes. A logistic regression modeling method with 'farm' variable as a random effect was used. The 'stage' variable was included as fixed effect into model in order to compare the presence of *Giardia* and *Cryptosporidium* among samples collected from manure pit, finishers, sows, and weaners samples.

Results

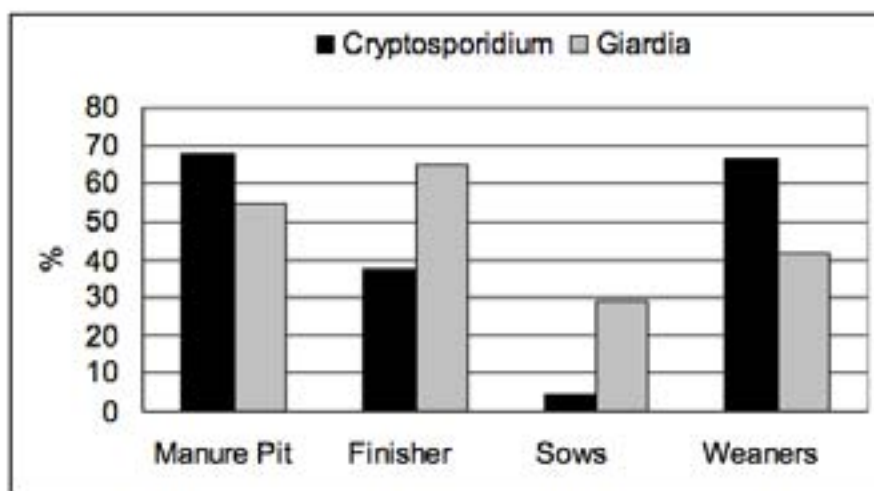
Cryptosporidium oocysts and *Giardia* cysts could be recovered at least from one sample on the all 10 farms which tested over the three visits of the study. *Cryptosporidium* oocysts and *Giardia* cysts were present in 62 (50.1%) and 54 (44.3%) of samples, respectively. However, using PCR, 81 (66.4%) and 68 (55.7%) of fecal samples were positive for *Giardia* and *Cryptosporidium*, respectively.

Prevalence of *Cryptosporidium* oocyst and *Giardia* cysts in manure pits did not differ ($P > 0.05$) (Figure 1). However, finisher pigs appeared to shed *Giardia* cysts (65%) less than *Cryptosporidium* oocyst (37%) ($P = 0.01$). Sows deemed to be tested positive more frequently for *Giardia* cysts (29%) versus *Cryptosporidium* oocyst (4.2%) ($P=0.02$). Weaners shed more *Cryptosporidium* oocyst (67%) compared to *Giardia* cysts (42%) ($P= 0.04$).

Unlike the prevalence of *Giardia* that has changed over the period of study, no significant change was observed in the prevalence of *Cryptosporidium* over the three visits. *Giardia* was present in 64.3% of samples collected during the first visit but it was isolated from 42.5% and 45.0% of the samples in visit 2 and visit 3, respectively. *Cryptosporidium* was isolated from 52.4%, 37.50%, and 42.50% of the samples collected in visit 1, visit 2, and visit 3, respectively.

Figure 1

Prevalence of *Giardia* and *Cryptosporidium* on fecal samples collected from manure pits, weaners, finishers, and sows



Cryptosporidium was more likely ($OR=3.6$) detected from manure pit samples and weaners ($OR=3.3$) compared to finisher pigs. However, it was less likely ($OR=0.06$) to be recovered from sows compared to finisher pigs. Prevalence of *Giardia* in samples collected from manure pits and finisher pigs did not differ ($P > 0.05$). However, *Giardia* had a decreased chance ($OR=0.2$) to be isolated from sows.

For *Cryptosporidium* spp., four different genotypes were determined; *C. parvum* (55.4%), pig genotype II (37.5%), *C. muris* (5.4%), and *C. suis* (1.8%) (Table 1). The two different *Giardia* genotypes were Assemblage B (92.1%) and Assemblage E (7.9%) (Table 2).

Table 1
Cryptosporidium genotypes in samples collected from finisher pigs, weaners, sows, and manure pits

| | No (%) of isolates | | | | |
|-------------------------------|--------------------|-----------------|--------------|-----------------|-----------------|
| | Manure pit | Finisher | Sows | Weaners | Total |
| <i>C. parvum</i> | 8 (62) | 12 (50) | 5 (100) | 6 (43) | 31 (55) |
| <i>C. sp. pig genotype II</i> | 2 (15) | 11 (46) | 0 (0) | 8 (57) | 21 (38) |
| <i>C. muris</i> | 3 (23) | 0 (0) | 0 (0) | 0 (0) | 3 (5) |
| <i>C. suis</i> | 0 (0) | 1 (4) | 0 (0) | 0 (0) | 1 (2) |
| Total | 13 (100) | 24 (100) | 5 (0) | 14 (100) | 56 (100) |

Table 2
Giardia genotypes in samples collected from finisher pigs, weaners, sows, and manure pits

| | No (%) of isolates | | | | |
|--------------|--------------------|-----------------|-----------------|-----------------|-----------------|
| | Manure pit | Finisher | Sows | Weaners | Total |
| Assemblage B | 14 (87) | 22 (92) | 10 (100) | 12 (92) | 58 (92) |
| Assemblage E | 2 (13) | 2 (8) | 0 (0) | 1 (8) | 5 (8) |
| Total | 16 (100) | 24 (100) | 10 (100) | 13 (100) | 63 (100) |

Discussion

A large proportion of the pooled swine manure samples tested in the present study showed the presence of *G. duodenalis* cysts and *Cryptosporidium* spp. oocysts. Similarly, high prevalences for both parasites have been previously reported in swine worldwide (Hamnes et al., 2007). However, the prevalence of both *G. duodenalis* and *Cryptosporidium* spp. infections in swine is believed to be age-specific. *Giardia* and *Cryptosporidium* have been reported commonly in different age groups of pig worldwide (Maddox-Hyttel et al., 2006; Xiao et al., 2004; Olson et al., 1997; Quilez et al., 1996) with higher prevalence among weaners than other age group (Maddox-Hyttel et al., 2006; Xiao et al., 1994).

The molecular characterization of *G. duodenalis* and *Cryptosporidium* spp. isolates from livestock is very useful to investigate possible sources of infection in human. In the present study, Assemblage B predominated in all swine age groups. Since both *G. duodenalis* Assemblage A and B are commonly reported in humans, the predominance of Assemblage B in the present study suggests that there may be a greater zoonotic potential for *G. duodenalis* than previously thought. *C. parvum* that is generally recognized as the major zoonotic species and rarely reported in pigs (Kvac et al 2009a), was common to all age categories suggesting a zoonotic risk from swine. The presence of *C. muris* in pig manure samples suggests the presence of rodents on the farm and the possible transmission between rodents and pigs (Chen and Huang, 2007).

Presence of *Cryptosporidium* and *Giardia* in stored manure warrants serious attention. The zoonotic transmission of *G. duodenalis* and *Cryptosporidium* spp. infections from swine to humans may occur through the contamination of surface water and produce. The manure management procedures may not be effective in order to eliminate the zoonotic agents from hog manure and as such fails to prevent the environmental contamination (Ziemer et al., 2010). Therefore, it is possible that those zoonotic agents get into ground water (Thurston-Enriquez et al, 2005) and contaminate the environment if spreading on land as fertilizer.

Conclusion

The occurrence of both *G. duodenalis* and *Cryptosporidium* spp. was high in the swine manure samples in the present study and zoonotic genotypes and species were identified. Therefore, further study will be required to demonstrate the source of these infections in swine, and molecular characterization of human isolates in this region would be required to investigate the actual risk of transmission to humans.

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