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

Farzan, A.[1]*
Dixon, B.[2], Pollari, F.[3], Friendship, R.[1], Cook, A.[3], Farber, J.[2], Parrington, L.[2], Pintar, K.[3]

[1] Department of Population Medicine, University of Guelph, Guelph ON CA;
[2] Microbiology Research Division, Bureau of Microbial Hazards, Health Canada, Ottawa ON CA,
[3] C-EnterNet Surveillance, Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph ON, CA

* Department of Population Medicine, University of Guelph, Guelph N1G 2W1 ON, Canada.
E-mail: afarzan@uoguelph.ca; Fax: +1 519 763 3117

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

Gasterointestinal 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 Cryptosboridium 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 Gairdia 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.

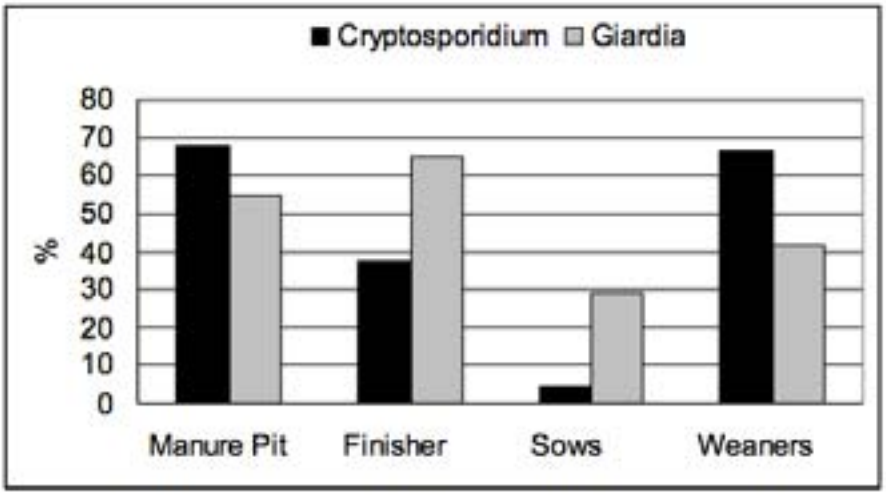
Results

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 Gairdia 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
C. parvum	8 (62)	12 (50)	5 (100)	6 (43)	31 (55)
C. sp. pig genotype II	2 (15)	11 (46)	0 (0)	8 (57)	21 (38)
C. muris	3 (23)	0 (0)	0 (0)	0 (0)	3 (5)
C. suis	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; Quìlez 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.

References

1. Chen F, and Huang K. 2007. Prevalence and phylogenetic analysis of Cryptosporidium in pigs in eastern China. Zoonoses Publ. Health.54:393-400.
2. Demczuk W, Ahmed R, Woodward D, Ahmed R, Clark C, Tabor H, Dore K, Ciampa N, and Muckle A. 2005. Laboratory Surveillance Data for Enteric Pathogens in Canada. Annual Summary 2002 and 2003.
3. Hamnes IS, Gjerde BK, Forberg T, and Robertson LJ. 2007. Occurrence of Cryptosporidium and Giardia in suckling piglets in Norway. Vet. Parasitol. 144:222-233.
4. Heitman TL, Frederick LM, Viste JR, Guselle NJ, Morgan UM, Thompson RC, and Olson ME. 2002. Prevalence of

5. Giardia and Cryptosporidium and characterization of Cryptosporidium spp. isolated from wildlife, human, and agricultural sources in the North Saskatchewan River Basin in Alberta, Canada. Can. J. Microbiol. 48:530-41.
6. Kosek M, Bern C, and Guerrant RL. 2003. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bulletin of the World Health Organization. 81:197–204.
7. Maddox-Hyttel C, Langkjær RB, Enemark HL, and Vigre H. 2006. Cryptosporidium and Giardia in different age groups of Danish cattle and pigs—occurrence and management associated risk factors, Vet. Parasitol. 141: 48–59.
8. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, and Tauxe RV. 1999. Food-related illness and death in the United States. Emerging Infectious Diseases. 5:607–625
9. Olson ME, O'Handley RM, Ralston BJ, McAllister TA, and Thompson RCA. 2004. Update on Cryptosporidium and Giardia infections in cattle. Trends. Parasitol. 20: 185–191.
10. Olson ME, Thorlakson CL, Deselliers L, Morck DW, and McAllister TA. 1997. Cryptosporidium and Giardia infections in Canadian farm animals. Vet. Parasitol. 68: 375–381.
11. Quìlez J, Sànches-Acedo C, Clavel A, Del Cacho E, and Lòpez-Bernad F. 1996. Prevalence of Cryptosporidium infections in pigs in Aragón (northeastern Spain). Vet. Parasitol. 67: 83–88.
12. Sanford SE. 1987. Enteric cryptosporidial infection in pigs: 184 cases (1081–1985). J. Am. Vet. Med. Assoc. 190: 695–69.
13. Thurston-Enriquez JA, Gilley JE, and Eghball B. 2005. Microbial quality of runoff following land application of cattle manure and swine slurry. J. Water. Health. 3:157-71.
14. Xiao L. 2010. Molecular epidemiology of cryptosporidiosis: an update. Exp. Parasitol. 124:80-9.
15. Xiao L, Herd RP, and Bowman GL. 1994. Prevalence of Cryptosporidium and Giardia infections on two Ohio pig farms with different management systems. Vet. Parasitol. 52: 331–336.
16. Ziemer CJ, Bonner JM, Cole D, Vinjé J, Constantini V, Goyal S, Gramer M, Mackie R, d Meng XJ, and Myers G, Saif LJ. 2010. Fate and transport of zoonotic, bacterial, viral, and parasitic pathogens during swine manure treatment, storage, and land application. J. Anim. Sci. 88: 84-94.