Article

Prevalence and genotypes of *Giardia duodenalis* in dairy and beef cattle in farms around Charlottetown, Prince Edward Island, Canada

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Abstract — Prevalence of *Giardia duodenalis* in dairy and beef cattle on farms around Charlottetown, Prince Edward Island (Canada) was determined by analyzing feces using direct immunofluorescence antibody microscopy. Genotypes were determined by 16S-rRNA sequencing. Fecal samples (n = 892) were collected from adult cattle in dairy tie-stall, dairy free-stall, and beef herds (10 herds each), and from calves (n = 183) from 11 dairy farms. Prevalence rates were 38% and 51% in cows and calves, respectively. *Giardia duodenalis* was present in all dairy herds, in 9/10 beef herds and in calves from 10/11 herds examined. Prevalence rates were 40% and 41% for cows in tie- and free-stall herds, respectively, and 27% for beef cows. Zoonotic Assemblage A was found in 12.2% of calves concomitantly infected with Assemblage E. All successfully sequenced samples (114/128) from cows corresponded to Assemblage E. *Giardia duodenalis* is highly prevalent in cattle herds in Prince Edward Island and Assemblage A in calves is a potential public health concern.

Résumé — Prévalence et génotypes de *Giardia duodenalis* dans les fermes laitières et bovines des environs de Charlottetown (Île-du-Prince-Édouard) au Canada. La prévalence de *Giardia duodenalis* chez les boivins laitiers et les bovins de boucherie dans les fermes des environs de Charlottetown, Île-du-Prince-Édouard (Canada) a été déterminée en analysant les fèces par immunofluorescence directe. Les génotypes ont été déterminés par le séquençage de l'ARNr 16S. Des échantillons de fèces (n = 892) ont été prélevés auprès du bétail adulte dans les stalles entravées laitières, les logettes laitières et les troupeaux bovins (10 troupeaux chacun) et de veaux (n = 183) provenant de 11 fermes laitières. Les taux de prévalence étaient de 38 % et de 51 % chez les vaches et les veaux, respectivement. *Giardia duodenalis* était présent dans tous les troupeaux laitiers, dans 9/10 troupeaux bovins et chez les veaux de 10/11 troupeaux examinés. Les taux de prévalence étaient de 40 % et de 41 % pour les vaches dans les stalles entravées et les logettes, respectivement, et de 27 % pour les vaches de boucherie. L'assemblage zoonotique A a été constaté chez 12,2 % des veaux avec une infection concomitante à l'assemblage E. Tous les échantillons séquencés avec succès (114/128) provenant des vaches correspondaient à l'assemblage E. *Giardia duodenalis* présente une prévalence élevée dans les troupeaux bovins de l'Île-du-Prince-Édouard et l'assemblage A chez les veaux constitue une préoccupation potentielle pour la santé publique.

(Traduit par Isabelle Vallières)

Can Vet J 2011;52:967–972

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Introduction

Giardia duodenalis is a common intestinal protozoan parasite that infects a wide variety of domestic and wild mammals as well as humans (1). Transmission of the parasite is dependent upon ingestion of cysts, which are excreted in the feces of infected hosts. While the direct fecal-oral route of transmission is important, waterborne transmission is a major route for human infections, specifically from *G. duodenalis* contaminated surface water (2,3). Typically, *G. duodenalis* infects the small intestine of the host. Clinical giardiasis in humans is recognized by diarrhea (acute or chronic), dehydration, abdominal pain, nausea, vomiting, and weight loss (4). Giardiasis in cattle is usually subclinical in adult cows. Calves may experience diarrhea; however, subclinical infections are common, probably depending on host, parasite, and environment interactions. Mixed infections with other protozoan and viral pathogens are common and may be responsible for clinical signs encountered (5). Production impacts of *G. duodenalis* have been demonstrated in lambs, but studies in calves failed to conclusively demonstrate an effect of infection on production (6–9).

Prevalence of *G. duodenalis* infection is high in cattle throughout the world and all age-groups can be infected (5,10–17). Two distinct genotypes of *G. duodenalis* are common in cattle: Assemblage A, which is also isolated from humans and other animals, and Assemblage E, which appears to be specific for hoofed livestock (18,19). Recently, *G. duodenalis* Assemblage B, commonly infecting humans, was detected in cattle (20), but it has yet to be determined how commonly this Assemblage occurs in cattle. While previous studies have found that over 80% of cattle are infected with Assemblage E, the presence of Assemblages A or B in cattle could be of concern as these assemblages are considered zoonotic (18,19). However, the zooanthroponotic potential of Assemblages A and B (transmission from humans to animals) must also be considered (21). Prince Edward Island (PEI) is Canada's smallest province (5684.39 km^2), but it has the highest human population density in Canada (22). There are 72 100 cattle on the island which is inhabited by about 141 000 people (23,24). A recent study found a mean *G. duodenalis* prevalence of 49% in the bovine teaching herd at the Atlantic Veterinary College (AVC) on PEI and 43% of microscopy positive samples analyzed by PCR were Assemblage A (25). The present study was undertaken to determine the prevalence of *G. duodenalis* and the distribution of its genotypes in adult dairy cows housed in tie-stalls and free-stalls, and its prevalence in beef herds and dairy calves.

Materials and methods

Study population

Mature beef cows (> 1 y) (10 farms), and adult dairy cows (> 2 y) housed in free-stalls (10 farms) and in tie-stalls (10 farms) were sampled once between February and July 2004. The farms were a convenience sample of herds located close to the AVC, Prince Edward Island. Thirty animals were sampled on each farm with the exception of 2 beef farms, 1 dairy-tie stall, and 1 dairy free-stall farm where only 28 animals could be sampled. In total, 892 cows were sampled. The predominant breed on the dairy farms was Holstein-Friesian. The beef cows were from cow/calf operations and were mainly cross-breeds (Herefords, Angus, and Simmental). Nine of the 10 beef herds were housed on pasture at the time of sampling, while 1 herd was confined to an enclosed area.

In addition, 183 calves (< 6 mo old) from 11 dairy farms were sampled (sample sizes on farms ranging from 7 to 27) once during May to August, 2003. The farms were a convenience sample of herds that were located close to the AVC and were willing to participate in the study.

Sample collection

Fecal samples from dairy cows and calves were collected from the rectum of each animal using an individual disposable latex glove. Fecal samples from beef cows on pasture were collected immediately after a cow had defecated. Wooden tongue depressors were used to scoop up the superficial layer of the feces in order to prevent contamination from the ground. Each sample was placed in a plastic specimen cup with a screw-on lid, labeled, and transported to the AVC within 2 h after collection. The samples were stored at 4°C and processed within 24 h.

Concentration of *Giardia duodenalis* cysts

Giardia duodenalis cysts were concentrated from fecal samples according to a previously published method (5). To increase the likelihood of detecting cysts in adult cows' feces the procedure was modified to accommodate a sample size of 20 g. Samples were filtered through 1 sheet of cheesecloth and layered over 15 mL (cows) or 5 mL (calves) of sucrose solution (specific gravity 1.13) in a clean 50 mL (cows) or 15 mL (calves) tube. Samples were centrifuged at 800 × *g* for 5 min, after which the interface and the upper layer of liquid were transferred, using a disposable pipette, to a clean 50 mL (cows) or 15 mL (calves) tube. The samples were then centrifuged again at 800 × *g* for 5 min and the supernatant was decanted, leaving a ~0.5-mL pellet.

After concentration, a 0.02-mL sample of the concentrate was spotted onto 1 well of a 2-well fluorescence microscopy slide (Erie Scientific, Portsmouth, New Hampshire, USA), and dried on a slide warmer at 37°C for approximately 10 min. A *Giardia*-specific fluorescein isothiocyanate (FITC)-labeled monoclonal antibody solution (0.04 mL) (Giardi-a-Glo; Waterborne, New Orleans, Louisiana, USA) was applied to the slide, which was then incubated in a humid air chamber for 40 min. After incubation, the slide was briefly rinsed with phosphate-buffered saline (pH 7.4) and allowed to completely air dry. A drop of mounting medium (AquaPolymount; Polysciences, Warrington, Pennsylvania, USA) was added to the slide which was then sealed with a glass cover slip. *Giardia duodenalis* cysts were examined and enumerated under an immunofluorescence microscope (Axioplan, Zeiss West Germany) at 100× magnification. Positive control slides containing formalin-fixed cysts (Giardi-a-Glo) and prepared as described above, were used to compare and confirm *G. duodenalis* cysts. One slide was examined for each sample. The number of cysts/g of feces was calculated using the following formula (5):

$$N = (s \times pv)/(vol \times wt)$$

where:

N = number of cysts/g of feces,
s = number of cysts counted on the slide,
pv = pellet volume (approximately 0.5 mL),
vol = volume of sample examined (0.02 mL),
wt = weight of fecal sample (g).

The minimum detection limit of this procedure is approximately 50 cysts/g of feces (25).

Molecular characterization

Polymerase chain reaction (PCR) was used to genotype positive samples. DNA was extracted from sucrose flotation concentrated cow feces using the QIAGEN DNeasy tissue kit (QIAGEN, Mississauga, Ontario). The manufacturer's protocol was adhered to with the following modifications: 50 μL of starting volume was used in step 1 and samples were lysed at 55°C overnight (step 2). To increase the concentration of recovered DNA, the nucleic acid was eluted in 100 μL of the supplied "Buffer AE" (10 mM Tris-Cl, 0.5 mM EDTA, pH 9.0). DNA was extracted from processed calf samples using the QIAGEN DNeasy tissue kit, following the manufacturer's instructions. Polymerase chain reaction (PCR) was performed on 292 (87.4%) of 334 cow samples that were positive for *G. duodenalis* by direct immunofluorescence antibody microscopy (DFA). Cow samples from all 29 *G. duodenalis*-positive farms were evaluated but not all DFA-positive samples from every farm were available for PCR testing. Seventy-six (80.9%) of 94 *G. duodenalis* DFA-positive calf samples from all 10 positive farms were available for PCR testing. Nested PCR was used to amplify a fragment of the *G. duodenalis* 16S-rRNA gene generating a 292 bp product (11). Polymerase chain reaction products were sequenced directly using the original amplification primers at McGill University and Genome Québec Innovation Centre (Montréal, Québec). Each PCR product was sequenced in both directions. Sequence chromatograms from each strand were aligned and inspected using the BioEdit Sequence Alignment Editor (version 7.0.9.0, available from http://www.mbio.ncsu.edu/bioedit/bioedit.html) (26). *Giardia duodenalis* genotypes were identified by comparison to GenBank sequences corresponding to *G. duodenalis* Assemblages A, B, C, D, E, F, and G. (GenBank accession numbers AF199446, DQ385547, DQ385548, DQ385549, AF113902, AF1994441, and AF1994501, respectively) (11,27,28), and by chromatogram analysis.

Data analysis

Giardia duodenalis prevalence for all cows and within different management groups of cows was estimated by a logistic regression model. Any clustering within herds was accounted for by the generalized estimating equation (GEE) procedure (29). A similar analysis was conducted for the calf data. Prevalence and 95% confidence interval (CI) were obtained by back-transformation of estimates from logistic to probability scale. To assess whether the prevalence between dairy tie-stall, dairy free-stall, and beef cow herds differed significantly, a Wald-test was performed. Cysts/g of feces excreted by cows and calves were naturally log-transformed and a Shapiro-Wilk test was used to test the normality of the distributions. A non-parametric Wilcoxon test was used to compare the geometric mean cyst count/g of feces between cows and calves.

A z-test using the estimates from the logistic regression model was carried out to assess whether the prevalence of *G. duodenalis* was significantly different between cows and calves. Cysts/g of feces of the calves were naturally log transformed and a Shapiro-Wilk test was used to test the normality of the distribution. All analyses were performed using the statistical software package STATA9.0 (Stata Corporation, College Station, Texas, USA). Values of $P \leq 0.05$ were considered statistically significant.

Table 1. Population-averaged prevalence of *Giardia duodenalis* in cows ($n = 892$) and calves ($n = 183$), and herd-level prevalence in dairy tie-stall ($n = 298$), dairy free-stall ($n = 298$) and beef herds ($n = 296$) in Prince Edward Island, Canada

	Prevalence (95% CI)[a]	Range within herds
Cows	0.38 (0.30–0.46)	0–0.79
Dairy tie-stall	0.40 (0.32–0.49)	0.17–0.57
Dairy free-stall	0.41 (0.29–0.53)	0.10–0.63
Beef	0.32 (0.18–0.51)	0–0.79
Calves	0.51 (0.37–0.66)	0–0.82

[a] Confidence interval.

Results

Giardia duodenalis prevalence and cyst count

The population-averaged cow- and calf-level prevalence rates of *G. duodenalis,* adjusted for clustering within herds, and the prevalence within dairy tie-stall, dairy free-stall, and beef herds, adjusted for clustering, are given in Table 1. There was no difference in *G. duodenalis* prevalence among the different cow management groups ($P = 0.72$). The prevalence rates between the cows and the calves were also not significantly different ($P = 0.1$). The herd-level prevalence rates of *G. duodenalis* were 100% (10/10) for dairy tie-stall and stall herds, and 90% (9/10) and 91% (10/11) for the beef herds and calves, respectively.

The naturally log transformed cyst counts/g of feces in cows and calves were not normally distributed (both $P < 0.001$). The geometric mean cyst counts/g of feces shed by the cows and calves were 11.4 [95% confidence interval (CI): 10 to 13] and 198.7 (95% CI: 123.9 to 318.6), respectively ($P < 0.001$).

Molecular characterization

The results from the molecular characterization of PCR-positive samples from calves are summarized in Table 2. The natural log-transformed cyst counts/g of feces were normally distributed ($P = 0.05$) and the geometric mean number of cysts/g of feces from calf samples with a positive PCR result (5.2 cysts/g of feces) was not significantly different from that of calves with a negative PCR result (5.0 cysts/g of feces) ($P = 0.54$). Calves with Assemblage E infection shed significantly higher numbers of cysts compared to calves carrying mixed Assemblage A and E infections ($P < 0.02$). Of the 292 cow samples available for genotyping, 128 (44%) were PCR-positive, representing all 29 farms on which *G. duodenalis* was identified by DFA.

Table 2. Genotypes (Assemblages) of *Giardia duodenalis* in dairy calves from 10 farms on Prince Edward Island, Canada

Farm	Number samples analyzed by PCR	Number positive in PCR	Assemblages isolated from PCR positive samples		
			A	E	A & E mixed
1	1	0 (0%)	0	0	0
2	14	6 (43%)	0	6	0
3	5	5 (100%)	0	4	1
4	13	8 (62%)	0	7	1
5	4	3 (75%)	0	3	0
6	7	4 (57%)	0	3	1
7	10	5 (50%)	0	5	0
8	13	7 (54%)	0	6	1
9	4	3 (75%)	0	2	1
10	5	0 (0%)	0	0	0
Total	76	41 (54%)	0 (0%)	36 (88%)	5 (12%)

PCR — polymerase chain reaction.

Eighty-nine percent (114/128) of samples were successfully sequenced and corresponded to Assemblage E. No Assemblage A was identified. There was an insufficient amount of feces to extract DNA from the other DFA-positive samples.

Discussion

The results from this study demonstrate that *G. duodenalis* is abundant in the studied dairy and beef herds in PEI. Although these results may not be extrapolated to all dairy and beef herds in PEI due to the convenience sampling frame, the prevalence of *G. duodenalis* in the herds examined here is within the prevalence ranges (4.5% to 89%) found in other studies (12–16, 30–34). Investigations from other parts of Canada also support the findings presented here. In Quebec, 46% of dairy farms sampled were infected with *G. duodenalis* and in Ontario, the overall prevalence of *G. duodenalis* in 669 beef cows was 9% (35,36). Beef cows sampled from farms in British Columbia, Alberta, and Saskatchewan had a prevalence of 17% (37). A *G. duodenalis* prevalence of 15% was reported in 20 beef dams in Alberta (38). Twenty-nine percent of 104 cattle from 6 geographic locations in Canada were infected with *G. duodenalis* (39).

In western Canada, the prevalence of *G. duodenalis* in beef calves ranged from 23% to 85% (11,36–38) with cumulative prevalence in longitudinal studies as high as 100% and prevalence rates in 2 studies in dairy calves were 57% and 73% (40,41). The prevalence in the calves from this study tended to be higher than in the adult cows, as in other studies (16,40,42). In the present study, the calves excreted significantly more cysts/g of feces than the cows, consistent with other reports (10,16,38). Most prevalence studies, including this one, estimate a point-prevalence. Because *G. duodenalis* cyst excretion can be intermittent, the actual prevalence is likely higher and has been underestimated in the present study (42). Furthermore, the minimum detection limit of the diagnostic test applied to the bovine fecal samples is approximately 50 cysts/g of feces. Thus, samples with lower cyst concentrations may have been falsely classified as negative. A lower concentration of *G. duodenalis* cysts could account for the lower prevalence observed in cows compared to calves, as cows produce larger quantities of feces, thus diluting the concentration of cysts excreted.

The zoonotic potential of *G. duodenalis* is becoming increasingly clear with the use of molecular techniques to genotype isolates (43). In this study, the zoonotic Assemblage A was present in calves but not in the cows. It is possible that immunologically mature cattle are able to resist infection with Assemblage A while the host-adapted Assemblage E is capable of establishing infection. Competition between genotypes within a host in favor of the host-adapted Assemblage has also been suggested (43). A significant association between genotype and cysts/g of feces was found in 1 study; however, this was only amongst different Assemblage E sequences isolated in that study (17). In the present study, calves with mixed infections shed significantly less cysts/g of feces compared to calves with only Assemblage E infection. However, calves with mixed infections were much less common.

The PCR methodology and primers employed in this study have been widely used (14,31,44) but there can be difficulties in amplifying and sequencing *G. duodenalis* DNA from bovine feces. In other studies, only a proportion of PCR positive samples was successfully genotyped (10,11,41). The potential presence of inhibitors, which are common in fecal samples, likely accounted for the failure to amplify and sequence a number of isolates in this study.

Results from this study are supported by other studies on commercial beef and dairy herds, which have shown that 80% to 100% of the *G. duodenalis* isolates are Assemblage E (11,17,31,41). Assemblage E appears to be limited to artiodactyls, being found in alpacas, goats, sheep, and pigs, in addition to cattle (45). It has been suggested that infected cattle pose a minimal threat to public health and that human infections likely originate from other humans (11,20,31,46–48). However, several studies demonstrate at least the potential of infected cattle to act as a source of infections in humans (3,12–14,21,25,33,49,50). Forty-three percent of adult cattle in the bovine teaching herd at the AVC, were infected with Assemblage A (25). van Keulen et al (3) found *G. duodenalis* Assemblages A and B in farm animals and prevalence determinations of *G. duodenalis* in preweaned, post-weaned and 1- to 2-year-old dairy cattle from the United States revealed that 15%, 7%, and 3%, respectively, were shedding Assemblage A (12–14). In Italy, *G. duodenalis* Assemblages A and B were isolated from 16 and 5 out of 24 calves, respectively (21). The same study also found a calf that had a mixed infection of *G. duodenalis* Assemblages A and E. Mixed infections have also been reported from Belgium (49). While Assemblage E was the most prevalent genotype in the calves from that study, mixed infections were more common (31%) compared with this study. Assemblages A and B have also been isolated from dairy calves in New Zealand (33). Assemblage B has also recently been isolated from dairy cattle in Ontario, Canada (50). In the present study, no Assemblage B was isolated. It is possible that the prevalence of this genotype in the environment is generally lower on Prince Edward Island. It is also important to consider the possibility that a genotype present in smaller numbers may go undetected due to the exponential nature of the PCR (49). In addition, zooanthroponotic transmission, where infected humans act as the source of *G. duodenalis* in cattle, must also be considered in cases where Assemblage A or Assemblage B isolates are detected in cattle.

This study has established that there is a high prevalence of *G. duodenalis* in cows and calves on Prince Edward Island. The presence of Assemblage A in 5 calves from 5 different dairy farms suggests that calves on Prince Edward Island should be considered a potential source of zoonotic *G. duodenalis* cysts. Only host-adapted *G. duodenalis* Assemblage E was found in the adult cows, suggesting that their role in transmitting giardiasis to humans is minimal.

Acknowledgments

The authors thank Cynthia Mitchell and Matthew Saab for valuable technical and laboratory support throughout the study. CVJ

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