Prevalence of *Giardia duodenalis* genotypes in adult dairy cows

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Abstract

The prevalence of *Giardia duodenalis* genotypes was determined in adult dairy cows. Fecal specimens were collected from two farms each in Vermont, New York, Pennsylvania, Maryland, Virginia, North Carolina, and Florida. Specimens, cleaned of fecal debris and concentrated using CsCl density gradient centrifugation, were subjected to PCR and DNA sequence analysis. The prevalence of *G. duodenalis* infection, ranged from 3% to 64%, with an average prevalence of 27% (144 positive cows out of 541 examined). DNA sequence analysis of the 16S rRNA gene revealed the presence of both Assemblage A and Assemblage E, *G. duodenalis*. Overall, Assemblage E was present in 25% of all animals tested and Assemblage A was present in 2% of the animals. As a percentage of *G. duodenalis* isolates, Assemblage E represented 94% and Assemblage A represented 6%. Although, most of the cows were infected with a genotype that is not known to be infectious for humans, adult cows on five farms did harbor varying levels of zoonotic Assemblage A, *G. duodenalis*. Therefore, although adult cows do not appear to be a significant source of human infectious cysts in the environment, the risk from this age group should not entirely be discounted.

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1. Introduction

*Giardia duodenalis* (syn. *G. lamblia*, *G. intestinalis*) is a commonly identified intestinal parasite of mammals, including humans. Human giardiasis, most often associated with drinking water, is frequently diagnosed in the United States (Lee et al., 2002). Molecular characterization has revealed seven major genotypes (assemblages) of *G. duodenalis* with differing host ranges (Monis et al., 1999, 2003; Thompson et al., 2000; Thompson and Monis, 2004). Assemblages A and B infect humans, cattle, and many other mammals; Assemblages C and D infect dogs, Assemblage E infects hoofed livestock, Assemblage F infects cats, and Assemblage G infects rats (Monis et al., 2003; Thompson and Monis, 2004).

Previous point prevalence studies have reported wide variation in the levels of infection in cattle, and longitudinal studies often report cumulative prevalences of 100% (Xiao and Herd, 1994; Olson et al., 1997a,b; Ruest et al., 1998; O’Handley et al., 1999; Ralston et al., 2003; Castro-Hermida et al., 2006; Gow and Waldner, 2006; Hamnes et al., 2006). Such prevalence data based on microscopic analysis is readily available; however, molecular prevalence data on *G. duodenalis* genotypes in cattle is generally lacking. Additionally, some studies that have used molecular analysis, only analyzed a subset of the positive samples. This data does not provide an accurate assessment of the actual prevalence of different genotypes. Surveys of dairy cattle in Canada, Australia, and the Netherlands have reported predominately Assemblage E, with lower levels of
Assemblage A (O’Handley et al., 2000; Huetink et al., 2001; Appelbee et al., 2003).

A recent longitudinal study of 30 adult dairy cows at a veterinary school in Canada (Uehlinger et al., 2006) revealed that on average 49% of the cows were positive for *G. duodenalis*. Of 14 isolates that were sequenced, 6 (43%) were Assemblage A and 8 (57%) were Assemblage E. Three multi-state prevalence studies for *G. duodenalis* in the eastern United States reported that pre-weaned calves (<2 months of age), post-weaned calves (2–12 months of age), and 1- to 2-year-old heifers were infected with both Assemblages E and A *G. duodenalis* (Trout et al., 2004, 2005, 2006). Although there was significant farm-to-farm variation, Assemblage E was found in some calves/heifers on all farms (Trout et al., 2004, 2005, 2006). Assemblage A, however, was detected in pre-weaned calves on 7 of 14 farms, in post-weaned calves on 9 of 14 farms, and in 1- to 2-year-old heifers on 10 of 14 farms (Trout et al., 2004, 2005, 2006). These previous studies on the prevalence of *G. duodenalis* genotypes in U.S. dairy cattle represented a regional sampling of over 1400 dairy animals aged 1 week to 2 years. Similar genotypic data are not available for adult dairy cows. The present study on adult dairy cows concludes a 4-year age group-related project investigating the prevalence of *G. duodenalis* genotypes in dairy cows over a multi-state area.

2. Materials and methods

2.1. Dairy farms

Two commercial dairy farms in Vermont, New York, Pennsylvania, Maryland, Virginia, North Carolina, and Florida were selected based on an expected availability of 30 adult milking cows for fecal sampling. All 14 farms were the same as those visited in 2004 in a study of 1- to 2-year-old dairy heifers (Trout et al., 2006) and included 11 of the same farms visited in 2002 and 2003 in studies of pre-weaned and post-weaned dairy calves (Trout et al., 2004, 2005).

2.2. Animals

Adult dairy cows (over 2 years of age) were randomly selected for sampling on each farm. Farms were visited between March and September. The number of animals sampled and their ages were dependent on the population on a given farm; the only age criteria was that the animals be at least 25 months old; no upper limit was established. The number of

Table 1

<table>
<thead>
<tr>
<th>State</th>
<th>Farm</th>
<th>Number of animals sampled</th>
<th>Number positive by PCR</th>
<th>Prevalence (%) of each assemblage in adult cows</th>
<th>Assemblage as a percentage of total <em>G. duodenalis</em> isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>E</td>
</tr>
<tr>
<td>Vermont</td>
<td>VT-2</td>
<td>40</td>
<td>12 (30%)</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>VT-3</td>
<td>28</td>
<td>18 (64%)</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>New York</td>
<td>NY-1</td>
<td>32</td>
<td>11 (34%)</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>NY-2</td>
<td>34</td>
<td>10 (30%)</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>PA-1</td>
<td>35</td>
<td>1 (3%)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>PA-3</td>
<td>42</td>
<td>15 (36%)</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>Maryland</td>
<td>MD-1</td>
<td>39</td>
<td>9 (23%)</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>MD-2</td>
<td>33</td>
<td>2 (6%)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Virginia</td>
<td>VA-2</td>
<td>40</td>
<td>6 (15%)</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>VA-3</td>
<td>37</td>
<td>16 (43%)</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>North Carolina</td>
<td>NC-3</td>
<td>46</td>
<td>9 (20%)</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>NC-4</td>
<td>42</td>
<td>7 (17%)</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Florida</td>
<td>FL-1</td>
<td>49</td>
<td>11 (22%)</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>FL-2</td>
<td>44</td>
<td>17 (38%)</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>541</td>
<td>144 (27%)</td>
<td>2</td>
<td>25</td>
</tr>
</tbody>
</table>
useful specimens obtained ranged from 28 to 49 per farm (Table 1).

2.3. Fecal sample collection and processing

Fecal samples were collected and processed as described by Trout et al. (2004, 2005, 2006). Briefly, feces were collected from each cow into plastic screw cap specimen cups and processed within 4 days of collection. Fifteen grams of feces were mixed with water, passed through a 45 μm screen, and then subjected to density gradient centrifugation using CsCl. The supernatant was aspirated from each sample and washed twice with dH₂O; the final pellet was suspended in 500 μl of dH₂O.

2.4. DNA extraction, PCR, and DNA sequence analysis

Molecular analysis was conducted as described in Trout et al. (2004, 2005, 2006). Briefly, total DNA was extracted from each cleaned fecal sample using a DNeasyTissue Kit (Qiagen, Valencia, CA), with a slight modification of the kit protocol. A total of 50 μl of processed feces were suspended in 180 μl of ATL buffer and thoroughly mixed. To this suspension, 20 μl of Proteinase K (20 mg/ml) was added; after the suspension was incubated overnight at 55 °C, 200 μl of AL buffer was added. The remaining protocol followed manufacturer’s instructions except that the nucleic acid was eluted in 100 μl of AE buffer to increase the quantity of recovered DNA.

A fragment of the ssu-rRNA (~292 bp) gene was amplified by PCR, and products were analyzed on 1% agarose gel with ethidium bromide staining. This amplicon differs by one nucleotide between Assemblages E and A.

PCR products were purified using EXO-SAP enzyme (USB Corporation, Cleveland, Ohio) and sequenced with the same PCR primers used with the original amplification in 10 μl reactions, Big Dye™ chemistries. Due to the large number of samples, direct sequencing of PCR products was chosen over cloning. All of the PCR positive products were sequenced once, in both directions, on an ABI3100 sequencer analyzer (Applied Biosystems, Foster City, CA). Sequence chromatograms from each strand were aligned and inspected using Lasergene software (DNASTAR, Inc., Madison, WI). This technique would detect mixed infections if the ratios of the two assemblages were roughly 50/50, otherwise only the predominate genotype would be detected.

3. Results

The number and location of cows infected with G. duodenalis as determined by PCR are shown in Table 1. Of 541 cows on 14 farms examined by PCR, 144 (27%) were G. duodenalis positive. The prevalence of G. duodenalis infection varied considerably across farms, with the lowest prevalence (3%) on PA-1 and the highest prevalence (64%) on VT-3. Overall, on 9 of the 14 farms greater than 20% of the adult cows were found to be infected with G. duodenalis.

The percentages of G. duodenalis genotypes found on each farm are presented in Table 1. Two genotypes were identified: Assemblage E, which has been reported only in hoofed-livestock, and Assemblage A, which is infectious for humans and a number of other mammals. Assemblage E was identified on all farms, whereas Assemblage A was identified on 5 of the 14 farms. The average prevalence of Assemblage E was 25%, ranging from 3% on PA-1 to 64% on VT-3. On farms where Assemblage A was present, the lowest prevalence was 2% on both PA-3 and FL-2 and the highest percentage was 9% on NY-2. Overall, Assemblage A was found in 2% of adult cows and represented 6% of the total G. duodenalis that was isolated.

4. Discussion

G. duodenalis infections were detected in adult dairy cows on all 14 farms examined, with prevalence by PCR ranging from 3% to 64%. This variation is consistent with previous point prevalence studies of Giardia spp. in cattle (Xiao, 1994; Xiao and Herd, 1994; Olson et al., 1997a,b; O’Handley et al., 1999, 2000; Trout et al., 2004, 2005, 2006; Castro-Hermida et al., 2006; Gow and Waldner, 2006; Hamnes et al., 2006). Because cyst excretion can be intermittent (Buret et al., 1990), point prevalence studies are likely to underestimate the actual number of infected animals present at any given time. Longitudinal prevalence studies commonly report 100% of the animals infected (Xiao and Herd, 1994; O’Handley et al., 1999). Thus, the point prevalence data presented herein likely underestimates the actual prevalence in these adult cows. Additionally, no technique is sufficiently sensitive to detect very low levels of cyst production, thus invariably false negatives occur, further underestimating the actual prevalence.

Sequence analysis of the 16S rRNA gene was performed for every PCR positive sample to determine the prevalence of G. duodenalis genotypes. Thus, in the current study, genotype data was obtained for 144 cattle samples. Zoonotic Assemblage A, G. duodenalis was...
detected in cows on 5 of the 14 farms. Livestock-
specific, Assemblage E, *G. duodenalis*, was detected in cows on all farms, with nine farms having exclusively
Assemblage E. Averaged across all farms, 2% of the animals harbored Assemblage A and 25% harbored
Assemblage E. On farms where Assemblage A was
detected, this genotype represented between 6% (FL-2)
and 30% (NY-2) of the total *G. duodenalis* isolates
(representing 2% and 9% of the animals respectively).
Similar variation among farms was seen in three
previous studies conducted by our laboratory. In pre-
weaned and post-weaned calves, and 1- to 2-year-old
heifers, Assemblage A, *G. duodenalis* was detected at
varying levels on 7, 9, and 10 of 14 farms studied,
respectively (Trout et al., 2004, 2005, 2006). Whereas
Assemblage A was absent on some farms, this genotype
represented greater than 20% of the total *Giardia*
isolates on other farms (Trout et al., 2004, 2005, 2006).

The previous studies indicated there was little
difference across age groups, in the prevalence of each
genotype. Zoonotic Assemblage A was found in 6%,
7%, and 3% of pre-weaned, post-weaned, and 1- to 2-
year-old animals, respectively (Trout et al., 2004, 2005,
2006); Assemblage E was found in 34%, 45%, and 33%
of pre-weaned, post-weaned and 1- to 2-year-old
animals, respectively (Trout et al., 2004, 2005, 2006).
The current study revealed that adult cows had a lower
total prevalence of *G. duodenalis*, as well as a lower
prevalence of both genotypes, 2% Assemblage A and
25% Assemblage E. There were no apparent changes in
management techniques on farms that had been visited
previously, thus these decreases are likely due to
changing host–parasite dynamics and possibly to cyst
excretion falling below detectable levels.

In studies of *Cryptosporidium* species and genotypes
(Santín et al., 2004; Fayer et al., 2006, 2007), pre-
weaned calves were the primary source of the zoonotic
species, *C. parvum*, whereas post-weaned calves, 1- to
2-year-old heifers, and adult cows harbored primarily
species and genotypes that were not infectious for
humans. Indeed, on 3 of 14 farms, no *Cryptosporidium*
spp. was detected in any adult animals (Fayer et al.,
2007). The situation appears to differ for *G. duodenalis*.
Zoonotic, Assemblage A, *G. duodenalis* was detected in
adult cows on 5 of 14 farms, and Assemblage E was
detected on all farms. Thus *G. duodenalis* infections
appear to be more persistent in adult populations than
*Cryptosporidium* spp. infections. Additionally, data
from our previous studies (Trout et al., 2004, 2005,
2006) and current study suggests that the prevalence
Assemblage A *G. duodenalis* is more variable than that
of *C. parvum*

A recent longitudinal study sampling only adult
animals over an 8-month period reported the average
prevalence of *G. duodenalis* to be 49% (Uehlinger et al.,
2006). This is higher than the average of 25% reported
in the current study, but point prevalence data is
expected to underestimate the actual prevalence.

The present study demonstrates substantial levels of
*G. duodenalis* infection in adult dairy cows. These
levels would appear to be sufficient to provide a
reservoir of infectious organisms to initiate disease in
neonatal calves. The prevalence of Assemblage A is
lower than that reported in other age groups (Trout et al.,
2004, 2005, 2006) and this genotype is present on fewer
farms. Thus, adult cows appear to represent a lower risk
to humans than younger animals. However, 4 years of
surveillance data indicate that, for unknown reasons,
there is considerable farm-to-farm variation in the
prevalence of Assemblage A. Uehlinger et al. (2006)
reported an even higher ratio of Assemblages A to E
(43–57%), than was seen in the current study (7% A to
93% E), however, only a subset of the positive samples
were genotyped. It does, however, appear that adult
dairy cows harbor varying levels of zoonotic, Assem-
lage A, *G. duodenalis*. Thus, even though the risk to
humans might be lower than that from younger animals,
adult cows should not be discounted as potential sources
of human infective cysts.

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**References**

Prevalence and genotyping of *Giardia duodenalis* from beef

Buret, A., denHollander, N., Wallis, P.M., Befus, D., Olson, M.E.,
Inf. Dis. 162, 231–237.

Castro-Hermida, J.A., Carro-Corral, C., Gonzalez-Warleta, M.,
Mezo, M., 2006. Prevalence and intensity of infection of *Cryp-
tosporidium* spp. and *Giardia duodenalis* in dairy cattle in
Health 53, 244–246.

Fayer, R., Santin, M., Trout, J.M., Greiner, E., 2006. Prevalence of
species and genotypes of *Cryptosporidium* found in 1–2 year
112.

idium* species and genotypes in mature dairy cattle on farms in
eastern United States compared with younger cattle from the same


