Veterinary Parasitology 185 (2012) 151–157

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Prevalence of *Giardia duodenalis* assemblages among dairy herds in the New York City Watershed

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ARTICLE INFO

Article history:
Received 17 January 2011
Received in revised form
13 September 2011
Accepted 20 September 2011

Keywords:
Prevalence
Giardia
Genotypes
Dairy
Watershed

ABSTRACT

A longitudinal herd-level study was carried out to determine the cumulative incidence of *Giardia duodenalis* infections in dairy cattle in the New York City Watershed. We also sought to assess the changes in infection pattern of animals diagnosed as shedding *Giardia* over time, determine risk factors that may be associated with *G. duodenalis* infections, and identify potentially zoonotic infections. A total of 2109 fecal samples were randomly collected from dairy cattle at 34 farms in the New York City Watershed on a seasonal basis. A total of 504 *Giardia*-positive samples were identified by zinc sulfate flotation. The overall cumulative incidence of *G. duodenalis* based on flotation results was 23.9% with 73.8% of all infections occurring in animals under 180 days of age (372/504). The intensity of infection ranged from 2 to 563,200 cysts/gram of feces. Cattle shedding *Cryptosporidium* spp. oocysts were twice as likely to shed *G. duodenalis* cysts in comparison to the animals that did not shed oocysts (1.81 95% CI 1.26–2.60 $p = 0.0012$). In the multivariate analysis, only the age of the animal and the presence of dogs on the farm were significantly associated with the likelihood of shedding *G. duodenalis*. DNA was extracted from positive samples and analyzed by polymerase chain reaction (PCR) of the *beta-giardin* and *triosephosphate isomerase* genes of *Giardia* spp. 304 samples were analyzed by PCR of which 131 were sequenced. 22.1% of sequenced samples were identified as assemblage A and 77.9% were identified as assemblage E. Interestingly, 100% of specimens identified as assemblage A were from calves under 84 days of age indicating that younger cattle are important reservoirs for potentially zoonotic assemblages of *G. duodenalis.*

1. Introduction

Giardia duodenalis is a protozoan parasite of important public health significance in both the developed and developing world. It is the most common intestinal protozoan, causing giardiasis in 200 million people worldwide each year (WHO, 1996). *Giardia duodenalis* has a wide host range parasitizing a variety of mammalian species (Thompson, 2000). Because of its wide host range, the zoonotic potential of *G. duodenalis* should not be neglected. Ingestion of contaminated food and water, particularly from rivers and streams, is of particular concern to public health since as few as 10 cysts may cause infection (Rendtorff, 1954).

Current molecular characterization of *G. duodenalis* assemblages has led to subdivision of the species into eight distinct assemblages (A–H). Humans are infected with assemblages A and B which can also infect wildlife, companion animals, and livestock (Cacciò et al., 2005). Assemblages C and D are specific to dogs, assemblage E to hoofed livestock (such as goats, sheep, pigs, and cattle), assemblage F to cats, assemblage G to rodents (Monis et al., 2003), and assemblage H in marine mammals (Lasek-Nesselquist et al., 2010).

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doi:10.1016/j.vetpar.2011.09.030

G. duodenalis has been found in cattle all over the world at prevalences as high as 100% from some sampled herds (Xiao and Herd, 1994; Olson et al., 1997; Gow and Waldner, 2006; Hamnes et al., 2006). Infections with *G. duodenalis* in calves tend to occur towards the end of the neonatal period, though our laboratory has reported calves as young as two days of age shedding *Giardia* cysts (Mark-Carew et al., 2010). Infected calves have been shown to shed high levels of cysts at intensities of 10^5–10^6 cysts per gram between 4 and 12 weeks of age (O'Handley et al., 1999; Ralston et al., 2003). Additionally, calves can excrete cysts for at least 100 days without a significant decline in excretion intensity (O'Handley et al., 1999).

Shedding of assemblages A, B, and E have been reported in dairy cattle. In a study done in New Zealand, assemblages A and B were present in the study population, however, assemblage E, the livestock-specific genotype, was not found (Winkworth et al., 2008a,b). Feng et al. (2008) reported 83% of *Giardia* positive specimens from 58 dairy cattle having the zoonotic assemblage A genotype while the remaining 17% had the livestock-specific assemblage E genotype. Trout et al. (2004, 2005, 2006, 2007) published a series of papers where they assessed the presence of *Giardia* assemblages in dairy cattle of different age groups on farms in seven states in the United States. They reported 15% of pre-weaned calves, 7% of post-weaned calves, 3% of heifers, and 2% of adult cows shedding assemblage A cysts while assemblage E was found in 45% of post-weaned calves, 33% of heifers, and 25% of adult cows. These studies illustrate the heterogeneity of the distribution *Giardia duodenalis* infections among dairy cattle of various ages and show that characterization of *Giardia* species is important in understanding the ecology of the organism and its zoonotic potential.

Recent studies have sought to identify external factors associated with *Giardia* infections in dairy cattle. Age, specific management practices, and environmental conditions have been shown to be significantly associated with infections (O'Handley et al., 1999; Wade et al., 2000; Gow and Waldner, 2006; Winkworth et al., 2008a,b; Mark-Carew et al., 2010). In the present study, we set out to determine the prevalence of *Giardia duodenalis* in the New York State Watershed in dairy cattle, assess the zoonotic potential of *G. duodenalis* found in our study population, and to identify risk factors associated with infections.

2. Materials and methods

2.1. Study design

In our longitudinal epidemiologic study, the target population was dairy herds in the New York City Watershed in Delaware County, New York. A total of 34 farms were recruited and agreed to participate in the study from September 2006 to May 2008. A stratified sampling design was developed to capture the potential variability in the likelihood of shedding *G. duodenalis* in a herd. A maximum number of 20 samples were randomly collected from each farm, 11 to be collected from calves (animals under 180 days of age) and 9 from adult cattle. Samples were taken from all available animals if less calves and adult cattle were on the farms. Any animal that was diagnosed as not shedding *Giardia* was resampled to determine its current infection status (if the animal was still present on the farm during subsequent sampling). The sampling scheme was stratified by season based on similar weather patterns to capture the potential variability in shedding due to the time of the year: Winter (November through March), Spring (April through June), and Summer (July through October).

2.2. Sample collection and analyses

Fecal samples were collected per rectum, put in collection tubes labeled with the animal's identification number and birth date (if available), and stored in frozen cold packs until transported to the laboratory where they were stored at 4 °C until processing. The fecal consistency was identified as one of the following: loose/normal, dry, runny, liquid, or bloody. All samples were processed using a standard quantitative zinc sulfate (specific gravity of 1.18) and sucrose (specific gravity of 1.33) centrifugation concentration flotation to recover *Giardia* cysts and *Cryptosporidium* oocysts, respectively. Ten grams of feces was weighed and dissolved in 150 mL of water. The solutions were sieved with tea strainers then centrifuged (first in water then zinc in sulfate or sucrose) in 15 mL tubes to approximately enumerate the number of (oo)cysts/gram feces. Microscopic examination was carried out using bright field and phase contrast microscopy. An animal was considered positive for infection based on the presence of cysts that were the correct morphology and size as *Giardia*. After microscopic examination, slides were placed in 50 mL tubes filled with water and were stored at 4 °C. To prepare specimens for DNA extraction, slides and cover slips were removed from the tubes, and the tubes were centrifuged at $3000 \times g$ for 15 min at 4 °C. The supernatant was poured off until 5 mL was left in each tube. The pellet was resuspended in water and transferred to 2 mL tubes for storage at −20 °C.

2.3. Data collection

Data on putative risk factors hypothesized to be associated with the likelihood of shedding *G. duodenalis* cysts were collected using a questionnaire. The questionnaires were completed by personal interview of each dairy farm owner/manager and included data on demographic (age, sex, breed of animal), geographic (location of the farm), and management practices (housing, bedding and presence of other animal species on the farm).

2.4. DNA extraction

DNA was extracted from the samples using the DNeasy Tissue Kit (Qiagen, Valencia, CA) with a modified protocol (Santín et al., 2009). 180 μL of ATL Buffer and 20 μL of Proteinase K were added to 1.5 mL tubes containing 50 μL of processed feces. Following an overnight incubation at 56 °C, the manufacturer's protocol was followed. The only exception was that 100 μL of nucleic acid was eluted using AE buffer instead of the suggested 200 μL.