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Short communication

Characterization of *Giardia duodenalis* infections in dogs in Trinidad and Tobago

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ABSTRACT

To our knowledge, the zoonotic potential of *Giardia duodenalis* has not been assessed in companion animals in Trinidad and Tobago. This report details the first attempt to evaluate the potential zoonotic risk of *G. duodenalis* in dogs and identify assemblages of *G. duodenalis* found in dog populations on both islands. Fecal samples were collected from free-roaming dogs and dogs at the Trinidad and Tobago Society for the Prevention of Cruelty to Animals from October 2010 to June 2011. A total of 168 samples were collected of which 104 samples were analyzed for the presence of *G. duodenalis* by PCR amplification of the *ssu-rRNA* gene with subsequent assemblage-typing. A subset of samples was also analyzed by ELISA. Twenty-six samples were positive for *G. duodenalis* by PCR for an overall prevalence of 25%. Four samples were identified as assemblage C (15.4%), 21 as assemblage D (80.8%), and one as assemblage E (3.8%). Puppies were four-times more likely to be infected with *G. duodenalis* than adult dogs (OR 4.61, 95% CI 1.73–12.2). There was a significant agreement between ELISA and PCR in the detection of the protozoa ($\kappa = 0.67$). We infer from our results that while the prevalence of *G. duodenalis* is relatively high in Trinidad and Tobago, the zoonotic risk of infection in humans is low since neither assemblage A nor B was identified in the study population.

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1. Introduction

Giardia is a widespread parasite associated with gastrointestinal disease of humans, wildlife, livestock, and companion animals. It is estimated that 200 million people worldwide, particularly, in Latin America, Africa, and Asia, are infected with *Giardia* each year. In developed countries, giardiasis is considered an emerging infectious disease due to its association with gastrointestinal disease from several water- and foodborne outbreaks (WHO, 1996).

The genus *Giardia* includes several species that infect numerous hosts, including mammals, amphibians and birds. *Giardia duodenalis* infects a wide host range of mammalian species. *G. duodenalis* is further divided into eight assemblages. Assemblages A and B are considered the potentially zoonotic strains since they infect several mammalian species including humans, wildlife, livestock and companion animals. Assemblage C and D infect domestic and wild canids, assemblage E infects hooved livestock, assemblage F infects cats, assemblage G infects rodents, and assemblage H infects seals (Feng and Xiao, 2011).

The zoonotic potential of *G. duodenalis* has been investigated in various mammalian species, particularly, dogs. While host-specific assemblages C and D are often cited,

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a growing number of reports show assemblages A and B in dog populations around the world (Paoletti et al., 2008; Cooper et al., 2010; Upjohn et al., 2010; Volotão et al., 2011; Berrilli et al., 2012). A study done in Germany showed assemblage A was the most prevalent strain found in dogs (Leonhard et al., 2007). A handful of community-based studies have shown assemblage A and B infection in humans and dogs living in the same locality (Hopkins et al., 1997; Traub et al., 2004; Inpankaew et al., 2007; Cooper et al., 2010). Overall infection rates in dogs differ based on many variables, including the composition of dog populations (owned/stray/kennel) and the type and sensitivity of the test used for diagnosis. Reports of giardiasis range from 0.1% in owned dogs to as high as 100% in kenneled dogs. Puppies, free-roaming dogs, and shelter dogs have been shown to be at higher risk for infection than adult dogs and owned dogs (Stehr-Green et al., 1987; Papini et al., 2005; Dubná et al., 2007; Mircean et al., 2012).

The present study was conducted to identify *G. duodenalis* infections in dogs in Trinidad and Tobago. The aims were to determine the prevalence among owned, shelter, and free-roaming dog populations, to identify risk factors associated with *Giardia* infections, to characterize strains of *G. duodenalis* present in dogs on both islands of the country, and to evaluate the agreement between ELISA and PCR in the identifying of this protozoan.

2. Materials and methods

2.1. Study design and sample collection

A cross-sectional study was carried out to address the stated objectives. The target population consisted of dogs in Trinidad and Tobago. The study population included stray dogs as well as dogs at both branches of the Trinidad and Tobago Society for the Prevention of Cruelty to Animals (TTSPCA) in St. James, Trinidad and Lowlands, Tobago. Any dog at the TTSPCA with a fecal specimen in the kennel at the time of the collection was included in the study. Demographic information about each dog was available on kennel doors. For stray dogs only information on gender, location of collection, approximate age (puppy or adult), and type of housing was available. Fecal samples were collected from 168 animals using a convenience sampling method over an eight-month period from October 2010 to June 2011. At the time of collection, samples were placed in zip-lock bags, labeled with the name of each dog, and stored on ice packs in a cooler. They were then stored short-term (1–2 days) at 4 °C prior to microscopy.

2.2. Sample analysis and microscopy

All samples were processed using a qualitative centrifugation concentration flotation technique with zinc sulfate (1.18 spg) and sucrose (1.30 sg) as the flotation media. Microscopic examination was conducted using bright-field microscopy. This technique facilitated identification of larger parasites such as helminth eggs and coccidia oocysts. Slides were rinsed with water and the washings were poured into 1.5 mL centrifuge tubes for storage and subsequent processing.

2.3. DNA extraction

DNA was extracted from 104 samples using the Qiagen Blood and Tissue Kit (Valencia, CA) following a slightly modified protocol. The only exception was that 100 μL of nucleic acid was eluted using AE buffer instead of the suggested 200 μL. DNA extracted samples were stored at −20 °C.

2.4. PCR and DNA sequencing

All DNA-extracted samples were analyzed using PCR as the primary method of diagnosis. The *ssurRNA* gene was amplified using a previously described nested PCR protocol (Hopkins et al., 1997; Read et al., 2002). PCR products were analyzed on 1% agarose gel with ethidium bromide staining. All positive PCR products were purified using Exonuclease I/Shrimp Alkaline Phosphatase (Exo-SAP-IT) (USB Corporation, Cleveland, OH) and sequenced in both directions in 18 μL reactions at the Cornell University Life Sciences Core Laboratories Center using the Applied Biosystems Automated 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Sequence chromatograms of each strand were aligned and examined with MEGA-4 software (2008). Nucleotide sequence searches were conducted using BLAST (http://www.ncbi.nlm.nih.gov/blast) to compare *Giardia*-positive samples with those previously deposited in Genbank.

2.5. ELISA

The Remel ProSpect *Giardia* Microplate Assay was used for antigenic detection of *Giardia*. ELISAs were conducted on diluted fecal samples to evaluate the sensitivity of ELISA given that the samples were subjected to conditions not recommended by the manufacturer's protocol. Samples were thawed, and cotton swabs were used to mix the liquid. Swabs were added to specimen dilution buffer, and the manufacturer's protocol was followed thereafter. Both visual observation and plate reader results were used to determine *Giardia* positive samples.

2.6. Statistical analyses

Results from PCR amplification of the *ssu-rRNA* gene were used to determine the prevalence of *Giardia*-positive samples (proportion of samples that tested positive by PCR out of all samples examined) from the study population. The significance of association between each potential risk factor and the likelihood of dogs shedding G. duodenalis in the feces was evaluated using univariate logistic regression. The magnitude of the association was quantified using the odds ratios (ORs) and interval estimates of the OR were computed using the 95% confidence interval. Backward stepwise logistic regression analysis was performed to assess the significance of association between the likelihood of shedding *G. duodenalis* and each of the