**Enterocytozoon bieneusi Genotypes in Dogs in Bogota, Colombia**

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**Abstract.** *Enterocytozoon bieneusi* was detected in dogs in Bogota, Colombia for the first time. Of 120 dogs, 15% were positive by polymerase chain reaction (PCR). Infected dogs ranged from 2 to 14 years of age, and more male than female dogs were infected with *E. bieneusi* (20% versus 5%). All PCR-positive specimens were sequenced and three genotypes were identified. A dog-specific genotype (PtEbIX) was identified in feces from 16 dogs. For two dogs, the nucleotide sequences obtained were genetically identical to the *E. bieneusi* Peru 5 and K genotypes previously reported as human pathogens. This is the first time that Peru 5 and K genotypes have been identified in dogs. This study confirms that dogs are infected with both human pathogenic and host-specific genotypes.

**INTRODUCTION**

Microsporidian infections in humans have been reported worldwide, and Microsporidia are recognized as emerging opportunistic pathogens of humans. *Enterocytozoon bieneusi*, the most common species reported in humans,1 is responsible for chronic diarrhea and a wasting diathesis in immunocompromised patients, such as in patients with acquired immunodeficiency syndrome and organ transplant recipients.2–8 This species also infects immunocompetent persons.9–12 The transmission routes and sources of human infections are not known. Animals are a likely source of human infections because microsporidia have been found in fecal specimens of many domestic and wild mammals, as well as birds.13–19

Molecular methods have shown considerable genetic variation among isolates of *E. bieneusi*. Analyses of ribosomal DNA internal transcribed spacer (ITS) sequences have identified more than 70 genotypes of *E. bieneusi*.15,16,20–22 Some of these genotypes have been recognized as host-specific, and others have been found to infect humans and animals, supporting the likelihood of zoonotic transmission.3,7,13,15,16,21 For example, in a recent study the *E. bieneusi* genotype Peru16 was detected in the feces of seven household guinea pigs and in the stool of a child living in the same house.23

To date, there is little information available on the prevalence of *E. bieneusi* in companion animals. There have been only relatively few studies of cats and dogs.13,19,21,24–26 Zoonotic genotypes of *E. bieneusi* have been identified in cats in Portugal and Colombia13,19 and in dogs in Portugal.19 Only 13 natural infections of dogs with *E. bieneusi* have been documented, and molecular information was included for only 9 of those dogs.19,24–26 The aim of this study was to determine the prevalence of *E. bieneusi* in dog feces using molecular methods and sequence analysis of the ITS region of ribosomal DNA to identify *E. bieneusi* genotypes.

**MATERIALS AND METHODS**

Fecal samples from 120 stray dogs (80 males and 40 females) from Bogota, Colombia were placed in plastic bags that were labeled, sealed, cooled, and shipped overnight to the U.S. Department of Agriculture laboratory in Beltsville, Maryland. Dogs ranged from days to years of age and were grouped as follows: < 6 months (n = 16), 6–12 months (n = 8), and > 2 years (n = 96) (Table 1). Feces were cleaned of debris by using CsCl to concentrate spores and total DNA was extracted from each CsCl-cleaned fecal specimen using a DNeasyTissue Kit (Qiagen, Valencia, CA) with a slightly modified protocol as described.27

Polymerase chain reaction (PCR) amplification was performed using a set of nested primers specific for *E. bieneusi* that amplified the ITS region as well as a portion of the flanking large and small subunit ribosomal RNA genes.20 The PCR products were subjected to electrophoresis in a 1% agarose gel and visualized by staining the gel with ethidium bromide. Negative and positive controls were included in all PCRs. Negative controls from the first PCR were amplified in a second reaction to check for low-level contamination.

All PCR-positive samples were purified using EXO-SAP enzyme (U.S. Biochemical Corporation, Cleveland, OH). Purified PCR products were sequenced on both strands with the same primers used for the secondary PCR amplification using Big DyeTM chemistry and an ABI3100 sequence analyzer (Applied Biosystems, Foster City, CA). Sequence chromatograms from each strand were aligned and inspected using the Lasergene software (DNASTAR Inc., Madison, WI). Sequences of these fragments were compared with sequences in the GenBank database by Basic Local Alignment Search Tool analysis (National Center for Biotechnology Information, Bethesda, MD). Nucleotide sequences of *E. bieneusi* isolates from dogs were deposited in GenBank under accession nos. EU650271 to EU650273.

The prevalence of *E. bieneusi* was compared between sexes of dogs. Fisher’s exact test was used to analyze the data and differences were considered significant when *P* < 0.05.

**RESULTS**

Of 120 dog fecal samples tested for the presence of *E. bieneusi*, 18 (15%) were positive (Table 1). *Enterocytozoon bieneusi* was detected only in adult dogs (16 of 80) (Table 1) that ranged from 2–14 years of age (Table 2). More male (16 of 80) than female (2 of 40) dogs were infected with *E. bieneusi* (*P* = 0.0320, by Fisher’s exact test) (Table 1).

Nucleotide sequence analysis of the ITS region showed three distinct genotypes of *E. bieneusi*. Of the 18 *E. bieneusi*
sequences that were generated, 16 isolates were identical to published nucleotide sequences from one dog from Portugal (genotype PtEb IX; GenBank accession no. DQ885585) and three dogs from Switzerland (GenBank accession no. AF059610). One isolate was identical to the published *E. bieneusi* ITS sequences from genotype K (also identified as genotypes IV, BEB5, and Peru2) isolated from humans, cattle, and cats (GenBank accession nos. AF267141, AF242478, AY371277, AY331009, DQ885579, and DQ836343). Another isolate was identical to the *E. bieneusi* ITS sequences from genotype Peru 5 (also identified as genotype WL11) isolated from humans, cats, and foxes (GenBank accession nos. AY371280, AY237219, and DQ836344). Mixed infections with more than one genotype of *E. bieneusi* were not detected.

### DISCUSSION

The present study confirms the presence of *E. bieneusi* in dogs from Colombia. A PCR assay showed that the overall prevalence of *E. bieneusi* in 120 dogs was 15% as assessed by examination of one fecal sample from each dog. However, the true prevalence might be higher because excretion of spores can be intermittent. In a survey conducted in Germany, 60 dogs were tested by nested PCR for *E. bieneusi* but none were positive. Only 13 other natural infections with *E. bieneusi* have been documented in dogs from Portugal, Spain, and Switzerland. The prevalence in Colombia is slightly higher than obtained by microscopy in Spain (10.8% and 11.7%) and by PCR in Switzerland (8.3%). Our results suggest that *E. bieneusi* could be a common parasite in dogs, one that has remained unrecognized because it is not possible to detect this parasite by routine coproscopic methods. It is unclear why infections were not observed in dogs less than two years of age. The prevalence was significantly higher in males than females (20% versus 5%). Higher prevalences in males than in females have been observed in fur-beariing wild mammals and cats but the underlying reason for this difference is not known.

The genetic analysis of the ribosomal DNA ITS of *E. bieneusi* from dogs showed three genotypes. The genotype PtEbIX (GenBank accession nos. DQ885585 and AF059610) was the most prevalent genotype identified in 16 (89%) of the 18 positive dogs. Until now this genotype had been identified only in dogs in Portugal and Switzerland. Although data on the epidemiology of *E. bieneusi* in animals are still sparse, our findings suggest that this genotype is dog specific. The other two genotypes found in the present study, Peru5 and K, have not been identified in dogs. Genotype Peru5 (also known as WL11) has been identified in humans in Peru, in a cat in Colombia, and in a fox in the United States (GenBank accession nos. AY371280, DQ836344, and AY237219), and genotype K (also identified as genotypes IV, PtEbIII, BEB5, and Peru2) has been identified in humans in Cameroon, France, Gabon, Germany, Niger, Peru, Uganda, and the United Kingdom, in cats in Colombia and Portugal, and in cattle in Portugal and in the United States (GenBank accession no. AF242478, AF267141, AY371277, DQ836343, DQ885579, and AY331009). Therefore, this is the first time that genotypes K and Peru5, which are considered zoonotic genotypes, have been found in dogs.

The current data indicate that dogs appear to be infected with human pathogenic and host-adapted genotypes. Our results also imply the existence of two distinct *E. bieneusi* populations cycling in dogs, with the dog-specific genotype appearing to have a dog-to-dog transmission and genotypes K, Peru5, Peru6, and Peru9 appearing to have the potential for zoonotic transmission from dogs to humans and vice versa. Although only a small portion of the *E. bieneusi* identified in dogs have the potential to infect humans, dogs could serve as a potential reservoir host for zoonotic *E. bieneusi* genotypes. Therefore, our findings strongly suggest that properly designed studies are needed to assess the role of dogs as a source of zoonotic microsporidiosis. On the basis of our results, dogs should be tested for *E. bieneusi*, especially in homes with high risk inhabitants such as immunocompromised persons.

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### REFERENCES


