Cryptosporidium muris in a Texas Canine Population

Philip J. Lupo, Rebecca C. Langer-Curry, Mary Robinson, Pablo C. Okhuysen, and Cynthia L. Chappell*

Center for Infectious Disease, The University of Texas School of Public Health, Houston, Texas; Center for Laboratory Animal Medicine and Care, and School of Medicine, The University of Texas Health Science Center at Houston, Houston, Texas

Abstract. Molecular technology has led to the discovery of previously unrecognized Cryptosporidium species in new hosts, such as C. canis in humans. The notion that dogs may transmit Cryptosporidium species to humans has significant public health implications, and additional studies are merited. The purpose of this study was to examine a group of kenneled dogs to determine the prevalence of Cryptosporidium species infection and to identify parasite species. Prevalence of active infection was 71%. Six positive samples were analyzed by polymerase chain reaction amplification of the 18S ribosomal RNA gene followed by restriction fragment length polymorphism analysis to identify the Cryptosporidium species. Restriction digest patterns identified C. muris as the infecting species in all six dogs; species identity was confirmed by genetic sequencing. To our knowledge, this is the first report of a naturally occurring C. muris infection in a canine host. The finding of C. muris in asymptomatic canines supports the notion of dogs as potential sources of human infection.

INTRODUCTION

Cryptosporidium species are intracellular, apicomplexan parasites that infect a wide variety of mammals. In humans, Cryptosporidium is a causative agent of diarrhea and is now recognized in more than 150 different host species throughout the world.1 Cryptosporidium is ubiquitous in human populations, and infection commonly results from exposure to contaminated water or an infected person or animal. Cryptosporidium is difficult to control because the oocysts are stable in the environment, resistant to standard methods of disinfection, and their small size (< 5 μm) makes them difficult to efficiently remove from water by filtration.2

Genotyping of oocysts isolated from infected hosts has confirmed multiple Cryptosporidium species, distinguished distinct genotypes within the various species, and identified new species of Cryptosporidium. In fact, Cryptosporidium species not previously recognized as pathogenic in humans have been found in both immunocompetent and immunocompromised humans. Although C. parvum and C. hominis are responsible for most human infections, C. baileyi, C. canis, C. felis, C. meleagridis, C. muris, and Cryptosporidium pig and cervine genotypes have been detected in humans.3 Most Cryptosporidium species develop in enterocytes, generally causing a self-limited diarrhea in immunocompetent persons. In contrast, C. muris infects the stomach lining and can become chronic, but manifesting few, if any, symptoms.4,5

Stray and domesticated dogs in many countries throughout the world harbor Cryptosporidium,6 although in many studies the particular Cryptosporidium species was not identified. To date, C. parvum and C. canis are the Cryptosporidium species associated with canines.7 However, experimental challenge of dogs with C. muris resulted in a persistent but asymptomatic infection.4,5 Cryptosporidium infections in canines are typically associated with low oocyst excretion and few, if any, clinical signs.5

The possibility that dogs may transmit Cryptosporidium to humans has significant public health implications, and additional studies using sensitive detection methods, particularly in the United States, are merited. The purpose of this study was to examine a group of kenneled dogs to determine the prevalence of Cryptosporidium infection in this cohort and to identify parasite species.

Several studies have ascertained the prevalence of Cryptosporidium in dogs. These studies were conducted in various parts of the world, each with different canine populations (stray versus kenneled) and diagnostic tests (Table 1). Prevalence estimates ranged from 0.0% to 7.4% (median = 1.0%) for less sensitive detection methods (carbol fuchsia stain, acid-fast stain, sugar flotation),9 and from 0.0% to 23% (median = 6.5%) with more sensitive methods (immunofluorescence assay [IFA], enzyme immunoassay [ELA], polymerase chain reaction [PCR]). Two canine studies conducted in the United States10,11 reported prevalences of 2.0% and 3.8%. Furthermore, a recent study has suggested the possible transmission of C. canis from a dog to children residing in the same household.12 A single seroprevalence study of 20 dogs indicated a high prevalence (80%) of antibodies to Cryptosporidium,13 but the small sample size made generalization to the canine population at large difficult.

MATERIALS AND METHODS

Sample population. The study population was a convenience sample of 70 kenneled dogs (1–3 years of age) from the University of Texas Health Science Center (UTHSC) Kennels located in Huntsville, Texas. Dogs included in the study population were housed in one of two wings of the UTHSC Kennels. The West Wing included adult dogs (n = 29) kenneled in the UTHSC facility from birth. The South Annex housed adult dogs (n = 41) that were retired from their duties as Texas Department of Criminal Justice (TDCJ) tracking dogs for prisons located throughout the state. These dogs typically remain in the kennel from two weeks to one year. All dogs were hounds, and the study population was equal in terms of sex (51% female and 49% male). Fifty-three percent of the dogs were unrelated, and among those that were, there were a total of 10 family groups.

The kennels are concrete and brick buildings surrounded by an open field. All dogs were housed in indoor/outdoor cages on concrete pads as either one to two dogs per cage and were cared for by residents of the Texas Department of Criminal Justice in Huntsville. Cages were adjacent to a corridor (i.e.,...
The presence of Cryptosporidium in dogs, as positive for samples provided by the manufacturer. Any value > 0.05 was defined as positive. The samples were analyzed according to the manufacturer's instructions. The samples were tested in duplicate with the ProSpecT Cryptosporidium Microplate Assay (Alexon-Trend, Ramsey, MN). The samples were thawed and DNA was isolated using QIAamp DNA Stool Mini Kit (Qiagen Inc., Valencia, CA) according to manufacturer's protocol. Isolated DNA was subjected to 18S ribosomal RNA (rRNA) PCRs-RFLP analysis as described. Briefly, DNA (10 μL) was amplified in a nested PCR, and products were incubated for one hour at 37°C with either 5′-TTCTAGAGCTATAGGGTG-3′ (forward primer) and 5′-CCCCATCCTCAGGAAAGGA-3′ (reverse primer). Species-specific primers used in the second reaction were 5′-GGAGGTGTGATTATTAGATAAA-3′ (forward primer) and 5′-AAGGAGTAAGGAC-ACCTCAG-3′ (reverse primer). Digested products were resolved by electrophoresis on a 1.5% agarose gel and visualized by staining with ethidium bromide. These products were compared with previously published PCR patterns generated under the same conditions. The sample with the most intense RFLP band was subjected to sequencing, and the PCR product was analyzed on an ABI 3100 capillary automated DNA sequencer (Applied Biosystems, Foster City, CA). Sequencing was in one direction and conducted using genomic DNA. The resulting nucleotide sequence was subjected to homology BLAST search against GenBank and aligned with various Cryptosporidium nucleotide sequences using the ClustalX program (ftp://ftp-igbmc.u-stasbg.fr/pub/ClustalX/). A neighbor-joining phylogenetic tree was generated by molecular evolutionary genetic analysis, version 2.1, based on the basis of evolutionary distances calculated with the Kimura two-parameter model.

### RESULTS

#### Prevalence of Cryptosporidium infections

Stool samples (n = 70) from 56 cages yielded 40 cages (71%) positive for Cryptosporidium antigen by EIA. Negative EIA values (i.e., those < 0.05) ranged from 0.026 to 0.047. Fifty-one samples were EIA positive with values ranging from 0.050 to 0.075, i.e., near the cutoff point. There were no discrepant results (positive versus negative) between duplicate measurements. The coefficient of variation for the duplicate measurements was 6.6%, which represented minimal within-subject variation. Analysis of the 70 individual samples from these cages yielded 49 positive for Cryptosporidium antigen (prevalence = 70%). All fecal samples were formed, and no gastrointestinal signs were noted in the dogs at the time of collection. The lack of clinical signs is consistent with previous studies assessing Cryptosporidium infection in dogs. Puppies bred in the facility were not assessed for infection but were also asymptomatic.

Stools from six infected dogs randomly selected for genotyping were also tested by IFA (Crypto-Glo, Waterborne, Inc.) to examine the size and shape of the oocysts. Labeled oocysts were morphologically consistent with those of *C. muris*. One wing of the kennel housed dogs that were retired from duties as tracking dogs for prisons located throughout the state. Given their assignment as tracking dogs, these animals were exposed to numerous sylvatic environments. Another wing housed breeding dogs. Some dogs were transferred between wings. Although the dogs did not have daily contact with one another (except for cage mates), they used some of the same run yards and had contact during breeding. Cryptosporidium prevalence estimates were similar for both wings.
Genotyping Cryptosporidium species. Stools from six infected dogs were randomly selected for PCR-RFLP analysis along with two C. muris-negative controls. The PCR products indicated that all samples contained the genus Cryptosporidium (Figure 1). To determine the Cryptosporidium species, amplicons were digested (Figure 1) and compared with previously published banding patterns unique to Cryptosporidium spp. The RFLP analysis using two restriction endonucleases (Vsp I and Ssp I) each yielded a pattern indicative of Cryptosporidium muris (Figure 2). Furthermore, the PCR product from canine SA39 was sequenced (GenBank accession no. EU549784), and a BLAST search showed that the 18S rRNA PCR fragment revealed greater than 99% identity with a known C. muris DNA fragment from the same locus. Sequencing was conducted using genomic DNA yielding a 707-basepair amplicon. The sequence from canine SA39 was compared with a known C. muris DNA fragment (C. muris mouse genotype gi3873253). Identity of the infecting Cryptosporidium species was further confirmed by neighbor-joining phylogenetic analysis, which indicated that the C. muris dog isolate clustered with previously reported C. muris isolates. A phylogenetic tree (Figure 3) was constructed from the canine SA39 sequence and 10 Cryptosporidium species/isolates included in the analysis: C. muris 1 (gi3873252), C. muris 2 (gi3873253), C. muris 3 (gi22128355), C. muris 4 (gi49359175), C. muris 5 (gi6997), C. parvum (gi7001), C. serpentis (gi5870846), C. meleagridis (gi5714421), C. felis (gi5714422), and C. canis (gi5714423).

DISCUSSION

To our knowledge, this is the first report of a natural infection of C. muris in canines. The large percentage of infected dogs in this kennel setting suggested a common source of exposure or transmission by fecal cross-contamination of the environment. Because the facility has no evidence of rodent infestation and dog food is kept in sealed plastic containers, direct transmission between cage mates as well as fecal contamination of the exercise area are the most likely sources of spread within the colony. Although there was no visible evidence of rodent infection, the cages were open, and accessible for rodent contamination. One may also speculate that tracking dogs were exposed to rodent droppings during their training or tracking activities, which often included access to wooded areas. Rodents have previously drawn attention as potential sources of Cryptosporidium for other host species. Another previous study described a C. muris outbreak in captive marsupials. In that outbreak, mouse feces in the captive breeding facility contained C. muris, thus leading the investigators to conclude that the marsupials acquired the infection through contaminated food or water.

The 71% prevalence of Cryptosporidium in kennel dogs was determined with a commercially available EIA that detects Cryptosporidium antigens in fecal samples. Positive results in canines with confirmed C. muris infections suggested significant cross-reactivity between the two Cryptosporidium species.
species and the usefulness of this assay in detecting *C. muris* infections. Other studies conducted in our laboratory showed that the EIA yielded positive results when purified, intact *C. muris* or *C. parvum* oocysts were used in the test (unpublished data). Furthermore, in a preliminary experiment, titration of *C. muris* oocysts yielded positive results for wells containing $1.6 \times 10^5$ or more oocysts, the same level of detection as *C. parvum*.

In the RFLP analysis, controls (*C. hominis* and *C. parvum*) displayed unique banding patterns compared with those from the canines. Stool samples (*n = 6*) that yielded the highest EIA absorbance values were selected for genetic testing. The PCR results from these dogs were the same and all were consistent with the previously published *C. muris* pattern. Thus, testing of samples from additional dogs was deemed unnecessary. Sequencing of the 18S rRNA PCR fragment from canine SA39 showed >99% identity with a known *C. muris* DNA fragment. Figure 3 also supports the species identity of the canine parasite. Furthermore, the isolate from canine SA39 groups with other *C. muris* isolates, indicating infection with *C. muris*. The RFLP patterns and sequencing indicate *C. muris* infection in the six canines, suggesting that this species was circulating in the dog population and responsible for positive EIA results.

Cryptosporidiosis in dogs seems to be a particularly mild infection, regardless of the *Cryptosporidium* species. Because *C. muris* infection in dogs appears to be asymptomatic, it would likely have gone unrecognized in the absence of this study. Interestingly, *C. muris* infection often leads to chronic infection with the parasite. Thus, it is likely that dogs have a persistent infection or may be re-infected from repeated exposures.

The risk of infection among the animal handlers at the TDCl facility is presently unknown. Further studies will be required to evaluate infections in the exposed workers and, if found, the parasite’s potential for transmission within the larger prison population. Experimental studies of *C. parvum* or *C. hominis* in humans has shown a low infectious dose with some isolates. If the same holds true for *C. muris*, it is possible that even a low level of infection in dogs may be a source of human exposure.

The zoonotic potential of *Cryptosporidium* and the possibility of human infection by companion animals have important implications, particularly in at-risk populations, such as those who are immunocompromised. This study should serve to stimulate future research into the *Cryptosporidium* species that are potentially pathogenic for humans, as well as the sources and risk factors important in transmission of cryptosporidiosis.

**REFERENCES**


