Short Report: Investigation of Potential Zoonotic Transmission of Cryptosporidiosis in Southern India

Priya Rajendran, Sitara Swarna Rao Ajampurr, Divya Chidambaram, Deepthi Kattula, Deva Prasanna Rajan, Honorine Ward, and Gagandeep Kang*

Department of Gastrointestinal Sciences, Christian Medical College, Vellore, India; Division of Geographic Medicine and Infectious Diseases, Tufts Medical Center, Boston, Massachusetts

Abstract. The common species and subgenotypes causing cryptosporidiosis were studied in 394 children and 627 animals with diarrhea in Vellore in southern India. Although no zoonotic strains were identified in 13 infected children, 1 of 12 infected animals had C. hominis, indicating the potential for cross-species transmission. This study also reports C. xiaoi for the first time in India.

Cryptosporidiosis is a common parasitic infection causing diarrhea in humans and animals. Although diarrhea in livestock results in economic loss, symptomatic and asymptomatic infections in animals have the potential for transmission and are a threat to public health. Cryptosporidium spp. are generally considered to be host specific. Cryptosporidium galli and C. baileyi are found predominantly in chickens, C. muris in rodents, C. canis in dogs, C. felis in cats, C. suis in pigs, and C. meleagris in turkeys; and in recent years, C. xiaoii in sheep and goats. In cattle, although C. parvum is seen in calves, C. bovis, C. ubiquitum, C. ryanae, and C. andersoni have been reported from heifers and adult cows. In humans, cryptosporidiosis is predominantly caused by C. hominis and C. parvum, with occasional reports of zoonotic species including C. meleagris, C. felis, and C. canis.

In India, there have been numerous studies of cryptosporidiosis in humans and a few in animals, but only one recent study from eastern India has investigated zoonotic transmission. In previous studies from Vellore, we identified C. hominis as the predominant species in children with diarrhea from the community and hospital.11,12 With differences in the distributions of subgenotypes depending on the study setting. In this study, we explored the potential for zoonotic transmission of cryptosporidiosis in this region by comparing cryptosporidial species in animals and children with diarrhea in the same geographic area.

Diarrheal samples from animals were collected from a veterinary clinic and several dairy farms near Vellore during February 2007–May 2008. At the dairy farms, diarrheal samples from cows alone were collected. At the veterinary clinic, samples from cows, buffaloes, bullocks, and goats were collected. Fecal samples were collected from children less than five years of age who were hospitalized during January 2003–May 2006 for diarrhea, defined as the passage of three or more watery stools in a 24-hour period. This study was approved by the Institutional Review Board of Christian Medical College, Vellore. Permission was obtained from the chief veterinarian and the dairy unit manager to collect animal samples. For samples from children enrolled in this study, informed consent was obtained from parents or guardian.

Animal fecal samples were subjected to proteinase K (2 μg/mL in 20 mM Tris, pH 7.5, 10 mM EDTA, 0.1% sodium dodecyl sulfate) digestion for one hour. A published extraction protocol was then carried out by using alkaline (1M KOH and dithithreitol), acid (25% HCl and 2 M Tris HCl), and treatment with phenol-chloroform, followed by extraction using the Qiamp DNA Stool Minikit (Qiagen, Valencia, CA). DNA from fecal samples of children was extracted by using the same kit without pre-treatment of samples.

Nested polymerase chain reaction (PCR) specific for the small subunit (SSU) ribosomal RNA locus was used to screen Cryptosporidium spp., followed by restriction fragment length polymorphism (RFLP) for species determination (http://cryptodb.org/cryptodb/) and Cpgg40/15 PCR-RFLP for subgenotyping by using previously published primers and protocols. Additionally, PCRs specific for the heat-shock protein 70 (HSP-70) and actin loci were conducted for C. bovis isolates identified by RFLP by using published primers. Samples with ambiguous PCR-RFLP results and representative HSP-70 and actin PCR products were sequenced (ABI PRISM 310 Genetic Analyzer; Applied Biosystems, Foster City, CA). Multiple sequence alignments were carried out using MUSCLE with sequences representative of different species’ subgenotypes, followed by phylogenetic analysis by using PhyML and tree construction by using TreeDyn software (Phylogen.y.fr software, version 2). All sequences from this study were deposited in GenBank under accession nos. HM627525–HM627531. A total of 627 samples from animals with diarrhea were collected, including 589 cows (25 were calves), 2 buffaloes, 11 bullocks, and 25 goats (11 were kids). The mean duration of diarrhea was 4.5 days for adult animals, 4 days for calves, and 3 days for goat kids. Twelve (1.9%) samples were positive for Cryptosporidium spp., by PCR. Among these samples, seven C. muris, three C. bovis, one C. parvum, and one C. hominis were identified by RFLP. However, sequencing of the SSU ribosomal RNA PCR product showed that banding patterns identified by RFLP as C. muris were C. andersoni and the banding patterns identified as C. bovis were C. xiaoii. Further sequencing and analysis of the actin and HSP-70 PCR products confirmed the isolates as C. xiaoii (Figure 1).

Cryptosporidium andersoni was identified in adult cows, and C. xiaoii was identified in one goat and two cows. Cryptosporidium parvum was also identified in a goat kid, and C. hominis was also identified in a cow. No cryptosporidia were identified in buffaloes or bullocks.

Most studies from India and other countries have documented C. parvum as the predominant species in calves; in
other regions, *C.bovis* has been found to predominate.5,7,9 *Cryptosporidium andersoni* is recognized as a major species in adult cattle.5,8,10 This finding is consistent with that of our study in which we detected *C. andersoni* in 7 adult cows. *C. andersoni* has also been reported in 3 of 2,414 patients with diarrhea,16 suggesting possible zoonotic transmission to humans.

This study is the first report of *C. xiaoi* in India and is also the first report of this species in a cow. Previous reports have documented the presence of diverse species of *Cryptosporidium* and human *C. xiaoi*, which were also reported in 3 of 2,414 patients with diarrhea,18 suggesting possible zoonotic transmission to humans.

**REFERENCES**

CRYPTOSPORIDIOSIS IN LIVESTOCK AND CHILDREN IN INDIA


