

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

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**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.<sup>1</sup> *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, *C. wrairi* in guinea pigs, and *C. meleagridis* in turkeys,<sup>2</sup> and in recent years, *C. xiaoi* in sheep and goats.<sup>3,4</sup> 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.<sup>5</sup> In humans, cryptosporidiosis is predominantly caused by *C. hominis* and *C. parvum*, with occasional reports of zoonotic species including *C. meleagridis*, *C. felis*, and *C. canis*.<sup>6</sup>

In India, there have been numerous studies of cryptosporidiosis in humans and a few in animals,<sup>7–9</sup> but only one recent study from eastern India has investigated zoonotic transmission.<sup>10</sup> In previous studies from Vellore, we identified *C. hominis* as the predominant species in children with diarrhea from the community and hospital,<sup>11,12</sup> 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.<sup>13</sup> 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).<sup>14</sup> 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<sup>15</sup> was used to screen *Cryptosporidium* spp., followed by restriction fragment length polymorphism (RFLP) for species determination (<http://cryptodb.org/cryptodb/>) and *Cpgp40/15* PCR-RFLP for subgenotyping<sup>16</sup> 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.<sup>3</sup> 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 (Phylogeny.fr software, version 2).<sup>17</sup> 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. xiaoi*. Further sequencing and analysis of the actin and HSP-70 PCR products confirmed the isolates as *C. xiaoi* (Figure 1).

*Cryptosporidium andersoni* was identified in adult cows, and *C. xiaoi* 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

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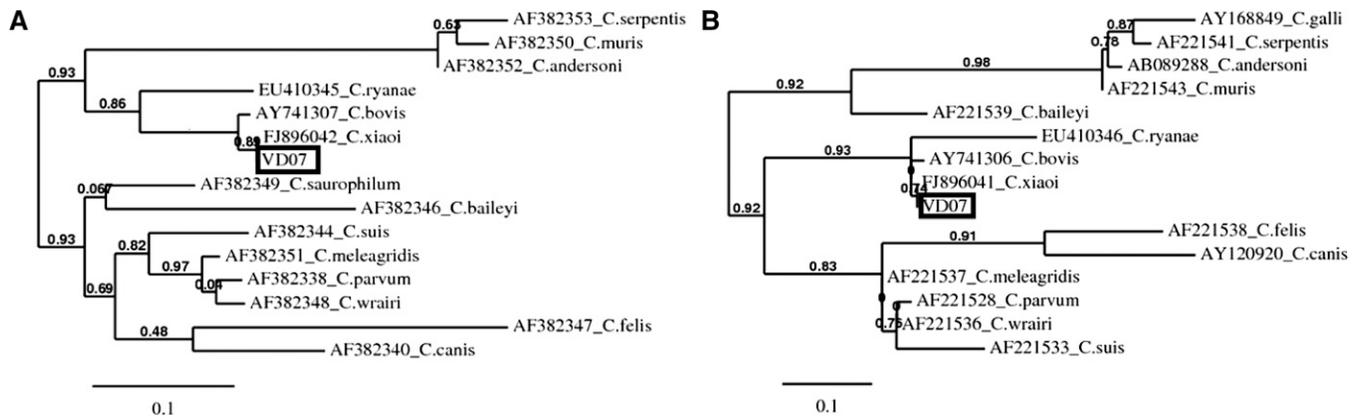


FIGURE 1. Phylogenetic analysis of cryptosporidial isolates at the **A**, actin locus (1,066 basepairs) with VD07 aligning with *Cryptosporidium xiaoi* (GenBank accession no. FJ896042, 98% identity at the nucleotide level), and the **B**, heat-shock protein 70 locus (325 basepairs) with VD07 aligning with *C. xiaoi* (GenBank accession no. FJ896041, 99% identity at nucleotide level). Scale bars indicate nucleotide substitutions per site.

other regions, *C. bovis* has been found to predominate.<sup>5,7,9</sup> *Cryptosporidium andersoni* is recognized as a major species in adult cattle.<sup>5,8,10</sup> 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,<sup>18</sup> 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 *C. xiaoi* in sheep, goats, and lambs.<sup>3,4</sup> When the *C. parvum* and *C. hominis* animal isolates were subjected to *Cpgp40/15* PCR-RFLP, only the *C. parvum* sample (VD 333 from a goat kid) could be subgenotyped and was identified as subgenotype IId. This result was further confirmed by sequencing as IIdA15G1. This subgenotype has been reported in humans, including a child with diarrhea from India, and could potentially be zoonotically transmitted.<sup>12,19</sup> Interestingly, a study from Spain has reported that the IId subtype is more common in goat kids than any other subgenotype.<sup>20</sup>

The *C. hominis* isolate that could not be genotyped was confirmed by sequencing the SSU ribosomal RNA PCR product (Genbank accession no. HM627527). This is the first report of *C. hominis* in a cow in India. A study from Scotland has also reported *C. hominis* from cows<sup>21</sup> and studies from other regions have reported this species from goats, sheep, and dugong, indicating that *C. hominis* may not be completely host restricted.<sup>22–24</sup>

Among the 394 samples from children hospitalized with diarrhea in the same geographic area, 13 (3.2%) were positive for *Cryptosporidium* by SSU ribosomal RNA PCR, all of which were *C. hominis*. Ten of the 13 positive samples could be subgenotyped (six as Ia, two as Ib, one as Ie, and one as Id). These circulating subgenotypes have been identified in studies from Vellore in hospital and community settings.<sup>11,12,25</sup>

This preliminary report identified a relatively low prevalence of cryptosporidial species in samples obtained from animal and human diarrhea. *Cryptosporidium andersoni*, which showed an RFLP pattern resembling that of *C. muris* but was identified by sequencing, was the predominant species in livestock,<sup>17</sup> and *C. hominis* was the predominant species in children.<sup>16</sup> *Cryptosporidium xiaoi* was reported for the first time in India in three animals.

Although no zoonotic species were identified in children in this study, studies in this area have identified *C. parvum*,

*C. felis*, and *C. meleagridis* among children with diarrhea and in human immunodeficiency virus-infected adults.<sup>25–27</sup> In the present study, detection of one *C. hominis* isolate in a bovine diarrheal sample and detection of *C. xiaoi* in a previously unreported animal species indicate that in an endemic setting, there is potential for cross-species transmission, including reverse zoonotic transmission. Although studies in northern India have documented the presence of diverse species of *Cryptosporidium* in cattle,<sup>7,8,10</sup> *C. hominis* has not been reported in animals in any studies in India. To address zoonotic transmission in more detail, ongoing longitudinal studies are being conducted in the community where animal–human contact is prolonged.

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