Molecular Characterization of *Cryptosporidium* Isolates from Humans and Animals in Iran

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Received 24 April 2006/Accepted 18 November 2006

Isolates of *Cryptosporidium* spp. from human and animal hosts in Iran were characterized on the basis of both the 18S rRNA gene and the Laxer locus. Three *Cryptosporidium* species, *C. hominis*, *C. parvum*, and *C. meleagridis*, were recognized, and zoonotically transmitted *C. parvum* was the predominant species found in humans.

*Cryptosporidium* is an apicomplexan parasite that infects humans and a wide range of domestic and wild animals. It is responsible for significant diarrheal diseases in both developing and developed nations. Molecular biology has provided powerful new tools for characterizing *Cryptosporidium* and has revealed considerable variation within the genus. Currently, 16 species are recognized (22), of which 7 infect susceptible immunocompetent and immunocompromised individuals. *C. parvum* and *C. hominis* are the species predominantly found in humans, but others, such as *C. meleagridis*, *C. felis*, *C. muris*, *C. canis*, and *C. suis*, have also been occasionally identified (3, 27).

*Cryptosporidium* has been previously reported in Iran (1, 10, 14, 17, 29), but apart from one documented case, in which a *C. parvum* infection was reported in the respiratory tract of an Iranian AIDS patient (14), no data are available on the molecular identification of the species infecting humans and animals in this country. Therefore, the present study was undertaken to identify *Cryptosporidium* species in human and animal hosts and to explore the transmission patterns of infection among them.

**Specimens, DNA isolation, and *Cryptosporidium* genotyping.**

Totals of 15 human and 9 animal stool specimens, collected from 2002 to 2005 in Iran and diagnosed positive for *Cryptosporidium* by acid-fast staining, were analyzed (Table 1). DNA was extracted using a QIAamp DNA stool kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. All specimens were genotyped on the basis of the 18S rRNA gene by nested PCR-restriction fragment length polymorphism (RFLP) (25, 26, 28) and sequencing (8). *Cryptosporidium* species were further confirmed by a Laxer sequence-based tool as previously described (9). Indeed, distinct Laxer PCR-RFLP patterns allowed the differentiation of *C. hominis*, *C. parvum*, and *C. meleagridis*, even distinguishing between two subgenotypes of *C. parvum*, as a result of DNA variation within this species (9).

**Cryptosporidium species identified.** DNA of all specimens yielded products of the expected 830-bp size by nested PCR of the 18S rRNA gene. Genotyping results from RFLP analysis of the amplified product were in agreement with those from DNA sequencing. The obtained 18S rRNA gene sequences matched the sequences previously deposited in GenBank. In the present study, *C. parvum* was identified in isolates from seven human immunodeficiency virus (HIV)-infected adults, four children, and seven cattle, whereas *C. hominis* was identified in isolates from one HIV-infected adult and three children. The third species, *C. meleagridis*, was identified in two turkey isolates (Table 1).

Results obtained by analysis of the Laxer DNA fragment were in agreement with those for the 18S rRNA gene locus (Table 1), except for three isolates in which, in spite of repeated attempts, DNA failed to amplify (the lower sensitivity of the PCR assay at the Laxer locus is the probable explanation). In human isolates, both the L1 and the L2 subgenotypes of *C. parvum* were recovered, while in cattle isolates, only the L1 subgenotype was found (Table 1).

**Cryptosporidium species in HIV-infected adults and in children.** Recent studies on cryptosporidiosis in HIV-infected adults and children in Iran have shown prevalences of 1.5% and 7%, respectively (10, 29). In the present study, the species responsible for cryptosporidiosis in Iranian patients were identified. Accordingly, in HIV-infected adults, *C. parvum* was more frequently identified than *C. hominis*. In contrast, in children, no significant difference in the distribution of *Cryptosporidium* species (*C. parvum* versus *C. hominis*) was observed. This pattern of *Cryptosporidium* species distribution in adults and children in Iran seems different from those in other...
acquired the pathogen by an anthroponotic pathway. Hypothesized that the adult infected by the H4-related isolate humans through anthroponotic transmission. Thus, it could be (5, 6, 20), were not found here. This work is also the first report bovis species reported to infect these animals, such as ridium species in animals. Cryptosporidium parasites had been reported in cattle in Iran (17), parvum infections in humans are the result of zoonotic trans-

TABLE 1. Isolates of Cryptosporidium genotyped in this study

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Host*</th>
<th>Cryptosporidium sp. identified by indicated method</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>Human (adult/HIV+)</td>
<td>C. parvum</td>
</tr>
<tr>
<td>H2</td>
<td>Human (adult/HIV+)</td>
<td>C. hominis</td>
</tr>
<tr>
<td>H3</td>
<td>Human (adult/HIV+)</td>
<td>C. parvum (L1 subgenotype)</td>
</tr>
<tr>
<td>H4</td>
<td>Human (adult/HIV+)</td>
<td>C. parvum</td>
</tr>
<tr>
<td>H5</td>
<td>Human (adult/HIV+)</td>
<td>C. parvum (L1 subgenotype)</td>
</tr>
<tr>
<td>H6</td>
<td>Human (adult/HIV+)</td>
<td>C. parvum (L2 subgenotype)</td>
</tr>
<tr>
<td>H7</td>
<td>Human (adult/HIV+)</td>
<td>C. parvum</td>
</tr>
<tr>
<td>H8</td>
<td>Human (adult/HIV+)</td>
<td>C. parvum (L1 subgenotype)</td>
</tr>
<tr>
<td>H9</td>
<td>Human (child)</td>
<td>C. hominis</td>
</tr>
<tr>
<td>H10</td>
<td>Human (child)</td>
<td>C. hominis</td>
</tr>
<tr>
<td>H11</td>
<td>Human (child)</td>
<td>C. hominis</td>
</tr>
<tr>
<td>H12</td>
<td>Human (child)</td>
<td>C. parvum (L1 subgenotype)</td>
</tr>
<tr>
<td>H13</td>
<td>Human (child)</td>
<td>C. parvum</td>
</tr>
<tr>
<td>H14</td>
<td>Human (child)</td>
<td>C. parvum (L1 subgenotype)</td>
</tr>
<tr>
<td>H15</td>
<td>Human (child)</td>
<td>C. parvum (L1 subgenotype)</td>
</tr>
<tr>
<td>C1</td>
<td>Cattle</td>
<td>C. parvum</td>
</tr>
<tr>
<td>C2</td>
<td>Cattle</td>
<td>C. hominis</td>
</tr>
<tr>
<td>C3</td>
<td>Cattle</td>
<td>C. parvum (L1 subgenotype)</td>
</tr>
<tr>
<td>C4</td>
<td>Cattle</td>
<td>C. parvum (L1 subgenotype)</td>
</tr>
<tr>
<td>C5</td>
<td>Cattle</td>
<td>C. parvum (L1 subgenotype)</td>
</tr>
<tr>
<td>C6</td>
<td>Cattle</td>
<td>C. parvum (L1 subgenotype)</td>
</tr>
<tr>
<td>C7</td>
<td>Cattle</td>
<td>C. meleagridis</td>
</tr>
<tr>
<td>T1</td>
<td>Turkey</td>
<td>C. meleagridis</td>
</tr>
<tr>
<td>T2</td>
<td>Turkey</td>
<td>C. meleagridis</td>
</tr>
</tbody>
</table>

* HIV+, HIV positive.

countries, such as Peru, Thailand, Malawi, Uganda, Kenya, South Africa, and South India, where C. hominis is by far dominant either in HIV-infected adults or in children (7, 11, 15, 18, 19, 23–25). However, in European countries, C. parvum is slightly more commonly identified than C. hominis in both immunocompetent and immunocompromised individuals (2, 4, 8, 13). Recently, C. parvum was also identified in children in Kuwait (21).

In the present study, among the C. parvum isolates, the L1 subgenotype was predominant, as it was identified in eight human cases out of nine and in all cattle cases. Interestingly, this subgenotype has also been the only one found in all cattle isolates from France and Tunisia, whereas both the L1 and the L2 subgenotypes were retrieved in humans from the same countries (K. Guyot, unpublished data). The failure to detect the L2 subgenotype in animals in the current study is in agreement with recent subtyping studies showing that not all C. parvum infections in humans are the result of zoonotic transmission (2, 12, 16). Indeed, this type of C. parvum would infect humans through anthropoноtic transmission. Thus, it could be hypothesized that the adult infected by the H4-related isolate acquired the pathogen by an anthropoноtic pathway.

Cryptosporidium species in animals. Prior to this work, Cryptosporidium parasites had been reported in cattle in Iran (17), but the present study reports the first molecular characterization of these protozoa in animals from this country. C. parvum has been the sole species identified in cattle. Other Cryptosporidium species reported to infect these animals, such as C. bovis, C. andersoni, and the Cryptosporidium deer-like genotype (5, 6, 20), were not found here. This work is also the first report of C. meleagridis infecting turkeys in Iran.

Conclusion. Few published reports on Cryptosporidium are available in the Middle East. In this study, despite the relatively small number of isolates characterized, the clear predominance of C. parvum in Iranian people might be considered the result of zoonotic transmission. However, more comprehensive epidemiological studies are needed to elucidate accurately the source of Cryptosporidium infection. Especially, further subtyping of C. parvum and C. hominis isolates using highly polymorphic markers is needed to improve our knowledge of parasite transmission pathways in Iran.

The Tehran University of Medical Sciences supported the Ph.D.-related training of A.R.M. in the Ecology of Parasitism Department at the Lille Pasteur Institute (Lille, France). The French Ministry of Research (quadrennial EA3609-Lille 2 University contract) supported this work.

We thank T. Ngouanesavan for her generous and enthusiastic cooperation.

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among Cryptosporidium isolates from human immunodeficiency virus-in-
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