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. suis, 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. Genotyping results from RFLP analysis of the 18S rRNA gene. Genotyping results from RFLP analysis 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).

Results obtained by analysis of the Laxer DNA fragment 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).

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).
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 protists 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. Ngouanesavanh for her generous and enthusiastic cooperation.

REFERENCES

rRNA gene of Cryptosporidium parasites from patients with or without hu-
man immunodeficiency virus infections living in Kenya, Malawi, Brazil, the
Nevez, J. C. Cailliez, D. Camus, and E. Dei-Cas. 2001. Molecular character-
ization of Cryptosporidium isolates obtained from humans in France. J. Clin.
Dei-Cas. 2002. PCR-restriction fragment length polymorphism analysis of a
diagnostic 452-base-pair DNA fragment discriminates between Cryptospori-
dium parvum and C. meleagridis and between C. parvum isolates of human
Analysis of sequence diversity at the highly polymorphic Cgpr40/15 locus
among Cryptosporidium isolates from human immunodeficiency virus-in-
Multilocus genotyping of Cryptosporidium parvum type 2: population genet-
ular epidemiological analysis of Cryptosporidium spp. in the United King-
dom: results of genotyping Cryptosporidium spp. in 1,705 fecal samples from
38:3984–3990.
and S. Solaymani-Mohammadi. 2006. Cryptosporidium parvum bovine geno-
type oocysts in the respiratory samples of an AIDS patient: efficacy of
treatment with a combination of azithromycin and paromomycin. Parasitol.
Res. 98:593–595.
15. Muthusamy, D., S. S. Rao, S. Ramani, B. Monica, I. Banerjee, O. C. Abra-
ham, D. C. Mathai, B. Primrose, J. Muliyil, C. A. Wanke, H. D. Ward, and
G. Kang. 2006. Multilocus genotyping of Cryptosporidium sp. isolates from
human immunodeficiency virus-infected individuals in South India. J. Clin.
Microbiol. 44:632–634.
Cryptosporidium population genetics: evidence of clonality in isolates from
19. Samie, A., P. O. Bessong, C. L. Obi, J. E. Sevilleja, S. Stroup, E. Houpt,
and R. L. Guerrant. 2006. Cryptosporidium species: preliminary descriptions of
the prevalence and genotype distribution among school children and hospital
patients in the Venda region, Limpopo Province, South Africa. Exp. Para-
Prevalence and age-related variation of Cryptosporidium species and geno-
22. Sunnotel, O., C. J. Lowery, J. E. Moore, J. S. Dooley, L. Xiao, B. C. Millar,
biol. 43:7–16.
68:710–715.
Cabrera, R. H. Gilman, and A. A. Lal. 2001. Identification of 5 types of
497.
26. Xiao, L., L. Escalante, C. Yang, I. Sulaiman, A. A. Escalante, R. J. Montali,
R. Fayer, and A. A. Lal. 1999. Phylogenetic analysis of Cryptosporidium
Microbiol. 65:1578–1583.
Rev. 17:72–97.
28. Xiao, L., U. M. Morgan, J. Limor, A. Escalante, M. Arrowood, W. Shulaw,
R. C. Thompson, R. Fayer, and A. A. Lal. 1999. Genetic diversity within
Cryptosporidium parvum and related Cryptosporidium species. Appl. Environ.
Microbiol. 65:3386–3391.
Mohraz. 2004. Prevalence of intestinal parasitic pathogens among HIV-