APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 2007, p. 1033–1035 Vol. 73, No. 3
0099-2240/07/$08.00+0 doi:10.1128/AEM.00964-06

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

Ahmad Reza Meamar,[1] Karine Guyot,[2] Gabriela Certad,[2] Eduardo Dei-Cas,[2] Mino Mohraz,[3] Mehdi Mohebali,[1] Kazem Mohammad,[4] Amir Ali Mehbod,[1] Sasan Rezaie,[1] and Mostafa Rezaian[1]*

Department of Medical Parasitology and Mycology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran[1]; Parasitology-Mycology Service, Microbiology Department, EA3609 Faculty of Medicine, Lille 2 University, University Hospital Centre and IFR-142, Lille Pasteur Institute, Lille, France[2]; Department of Infectious Diseases, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran[3]; and Department of Biostatistics and Epidemiology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran[4]

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

* Corresponding author. Mailing address: Department of Medical Parasitology and Mycology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, P.O. Box 14155-6446, Tehran, Iran. Phone: 98 21 88951392. Fax: 98 21 66462267. E-mail: rezaiian@sina.tums.ac.ir.

▽ Published ahead of print on 1 December 2006.

TABLE 1. Isolates of *Cryptosporidium* genotyped in this study

Isolate code	Host[a]	*Cryptosporidium* sp. identified by indicated method	
		18S rRNA gene sequencing	Laxer sequencing
H1	Human (adult/HIV+)	*C. parvum*	*C. parvum* (L1 subgenotype)
H2	Human (adult/HIV+)	*C. hominis*	*C. hominis*
H3	Human (adult/HIV+)	*C. parvum*	*C. parvum* (L1 subgenotype)
H4	Human (adult/HIV+)	*C. parvum*	*C. parvum* (L2 subgenotype)
H5	Human (adult/HIV+)	*C. parvum*	*C. parvum* (L1 subgenotype)
H6	Human (adult/HIV+)	*C. parvum*	No DNA amplification
H7	Human (adult/HIV+)	*C. parvum*	*C. parvum* (L1 subgenotype)
H8	Human (adult/HIV+)	*C. parvum*	No DNA amplification
H9	Human (child)	*C. parvum*	*C. parvum* (L1 subgenotype)
H10	Human (child)	*C. hominis*	*C. hominis*
H11	Human (child)	*C. hominis*	*C. hominis*
H12	Human (child)	*C. parvum*	*C. parvum* (L1 subgenotype)
H13	Human (child)	*C. hominis*	*C. hominis*
H14	Human (child)	*C. parvum*	*C. parvum* (L1 subgenotype)
H15	Human (child)	*C. parvum*	*C. parvum* (L1 subgenotype)
C1	Cattle	*C. parvum*	No DNA amplification
C2	Cattle	*C. parvum*	*C. parvum* (L1 subgenotype)
C3	Cattle	*C. parvum*	*C. parvum* (L1 subgenotype)
C4	Cattle	*C. parvum*	*C. parvum* (L1 subgenotype)
C5	Cattle	*C. parvum*	*C. parvum* (L1 subgenotype)
C6	Cattle	*C. parvum*	*C. parvum* (L1 subgenotype)
C7	Cattle	*C. parvum*	*C. parvum* (L1 subgenotype)
T1	Turkey	*C. meleagridis*	*C. meleagridis*
T2	Turkey	*C. meleagridis*	*C. meleagridis*

[a] 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 anthroponotic transmission. Thus, it could be hypothesized that the adult infected by the H4-related isolate acquired the pathogen by an anthroponotic 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 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

1. **Ahourai, P., A. Ezzi, M. R. Gholami, J. Vandyoosefi, R. Kargar, and N. Maalhagh.** 1985. *Cryptosporidium* spp. in new born lambs in Iran. Trop. Anim. Health Prod. **17:**6–8.
2. **Alves, M., L. Xiao, I. Sulaiman, A. A. Lal, O. Matos, and F. Antunes.** 2003. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. J. Clin. Microbiol. **41:**2744–2747.
3. **Caccio, S. M.** 2005. Molecular epidemiology of human cryptosporidiosis. Parasitologia **47:**185–192.
4. **Chalmers, R. M., K. Elwin, A. L. Thomas, and D. H. Joynson.** 2002. Infection with unusual types of *Cryptosporidium* is not restricted to immunocompromised patients. J. Infect. Dis. **185:**270–271.
5. **Fayer, R., M. Santin, J. M. Trout, and E. Greiner.** 2006. Prevalence of species and genotypes of *Cryptosporidium* found in 1-2-year-old dairy cattle in the eastern United States. Vet. Parasitol. **135:**105–112.
6. **Fayer, R., M. Santin, and L. Xiao.** 2005. *Cryptosporidium bovis* n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (Bos taurus). J. Parasitol. **91:**624–629.
7. **Gatei, W., J. Greensill, R. W. Ashford, L. E. Cuevas, C. M. Parry, N. A. Cunliffe, N. J. Beeching, and C. A. Hart.** 2003. Molecular analysis of the 18S

rRNA gene of *Cryptosporidium* parasites from patients with or without human immunodeficiency virus infections living in Kenya, Malawi, Brazil, the United Kingdom, and Vietnam. J. Clin. Microbiol. **41:**1458–1462.
8. **Guyot, K., A. Follet-Dumoulin, E. Lelievre, C. Sarfati, M. Rabodonirina, G. Nevez, J. C. Cailliez, D. Camus, and E. Dei-Cas.** 2001. Molecular characterization of *Cryptosporidium* isolates obtained from humans in France. J. Clin. Microbiol. **39:**3472–3480.
9. **Guyot, K., A. Follet-Dumoulin, C. Recourt, E. Lelievre, J. C. Cailliez, and E. Dei-Cas.** 2002. PCR-restriction fragment length polymorphism analysis of a diagnostic 452-base-pair DNA fragment discriminates between *Cryptosporidium parvum* and *C. meleagridis* and between *C. parvum* isolates of human and animal origin. Appl. Environ. Microbiol. **68:**2071–2076.
10. **Hamedi, Y., O. Safa, and M. Haidari.** 2005. *Cryptosporidium* infection in diarrheic children in southeastern Iran. Pediatr. Infect. Dis. J. **24:**86–88.
11. **Leav, B. A., M. R. Mackay, A. Anyanwu, R. M. O'Connor, A. M. Cevallos, G. Kindra, N. C. Rollins, M. L. Bennish, R. G. Nelson, and H. D. Ward.** 2002. Analysis of sequence diversity at the highly polymorphic *Cpgp40/15* locus among *Cryptosporidium* isolates from human immunodeficiency virus-infected children in South Africa. Infect. Immun. **70:**3881–3890.
12. **Mallon, M. E., A. MacLeod, J. M. Wastling, H. Smith, and A. Tait.** 2003. Multilocus genotyping of *Cryptosporidium parvum* type 2: population genetics and sub-structuring. Infect. Genet. Evol. **3:**207–218.
13. **McLauchlin, J., C. Amar, S. Pedraza-Diaz, and G. L. Nichols.** 2000. Molecular epidemiological analysis of *Cryptosporidium* spp. in the United Kingdom: results of genotyping *Cryptosporidium* spp. in 1,705 fecal samples from humans and 105 fecal samples from livestock animals. J. Clin. Microbiol. **38:**3984–3990.
14. **Meamar, A. R., M. Rezaian, S. Rezaie, M. Mohraz, E. B. Kia, E. R. Houpt, and S. Solaymani-Mohammadi.** 2006. *Cryptosporidium parvum* bovine genotype 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. Abraham, 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.
16. **Ngouanesavanh, T., K. Guyot, G. Certad, Y. Le Fichoux, C. Chartier, R. I. Verdier, J. C. Cailliez, D. Camus, E. Dei-Cas, and A. L. Banuls.** 2006. *Cryptosporidium* population genetics: evidence of clonality in isolates from France and Haiti. J. Eukaryot. Microbiol. **53:**533–536.
17. **Nouri, M., and R. Toroghi.** 1991. Asymptomatic cryptosporidiosis in cattle and humans in Iran. Vet. Rec. **128:**358–359.
18. **Peng, M. M., S. R. Meshnick, N. A. Cunliffe, B. D. Thindwa, C. A. Hart, R. L. Broadhead, and L. Xiao.** 2003. Molecular epidemiology of cryptosporidiosis in children in Malawi. J. Eukaryot. Microbiol. **50**(Suppl.)**:**557–559.
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. Parasitol. **114:**314–322.
20. **Santin, M., J. M. Trout, L. Xiao, L. Zhou, E. Greiner, and R. Fayer.** 2004. Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. Vet. Parasitol. **122:**103–117.
21. **Sulaiman, I. M., P. R. Hira, L. Zhou, F. M. Al-Ali, F. A. Al-Shelahi, H. M. Shweiki, J. Iqbal, N. Khalid, and L. Xiao.** 2005. Unique endemicity of cryptosporidiosis in children in Kuwait. J. Clin. Microbiol. **43:**2805–2809.
22. **Sunnotel, O., C. J. Lowery, J. E. Moore, J. S. Dooley, L. Xiao, B. C. Millar, P. J. Rooney, and W. J. Snelling.** 2006. *Cryptosporidium*. Lett. Appl. Microbiol. **43:**7–16.
23. **Tiangtip, R., and S. Jongwutiwes.** 2002. Molecular analysis of *Cryptosporidium* species isolated from HIV-infected patients in Thailand. Trop. Med. Int. Health **7:**357–364.
24. **Tumwine, J. K., A. Kekitiinwa, N. Nabukeera, D. E. Akiyoshi, S. M. Rich, G. Widmer, X. Feng, and S. Tzipori.** 2003. *Cryptosporidium parvum* in children with diarrhea in Mulago Hospital, Kampala, Uganda. Am. J. Trop. Med. Hyg. **68:**710–715.
25. **Xiao, L., C. Bern, J. Limor, I. Sulaiman, J. Roberts, W. Checkley, L. Cabrera, R. H. Gilman, and A. A. Lal.** 2001. Identification of 5 types of *Cryptosporidium* parasites in children in Lima, Peru. J. Infect. Dis. **183:**492–497.
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* parasites based on the small-subunit rRNA gene locus. Appl. Environ. Microbiol. **65:**1578–1583.
27. **Xiao, L., R. Fayer, U. Ryan, and S. J. Upton.** 2004. *Cryptosporidium* taxonomy: recent advances and implications for public health. Clin. Microbiol. 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.
29. **Zali, M. R., A. J. Mehr, M. Rezaian, A. R. Meamar, S. Vaziri, and M. Mohraz.** 2004. Prevalence of intestinal parasitic pathogens among HIV-positive individuals in Iran. Jpn. J. Infect. Dis. **57:**268–270.