Reevaluating the Molecular Taxonomy: Is Human-Associated *Cyclospora* a Mammalian *Eimeria* Species?

Human-associated *Cyclospora* is a coccidian parasite that causes diarrheal disease. A reevaluation of the parasite's molecular taxonomy that takes into account newly published data for seven *Eimeria* species shows that *Cyclospora* belongs to the *Eimeria* clade (Eimeriidae family). The *Cyclospora* branch on the phylogenetic tree is between the branches of the eight avian and two mammalian *Eimeria* species that have been evaluated to date. Furthermore, preliminary results indicate that *Cyclospora* and *Isospora belli*, another coccidian parasite that causes diarrheal disease in humans, belong to different families. To improve our understanding of the taxonomy of human-associated *Cyclospora*, molecular evaluation of isolates of additional *Cyclospora* and *Eimeria* species is needed.

In 1996 and 1997, human-associated *Cyclospora,* a protozoan apicomplexan parasite, caused outbreaks of diarrheal disease in the United States and Canada that were associated with consumption of various types of fresh produce (1,2). Human-associated *Cyclospora* was previously referred to as "cyanobacteriumlike body" or "coccidialike body" and "big (or large) *Cryptosporidium* " (3). In 1993, Ortega et al. (3) proposed, on the basis of morphologic and sporulation characteristics, that this parasite be placed in the genus *Cyclospora* (Schneider, 1881) in the coccidian family Eimeriidae (Minchin, 1903). Although the species designation *Cyclospora cayetanensis* was given in 1994 to Peruvian isolates of human-associated *Cyclospora* (4), it is not yet known whether all human *Cyclospora* isolates belong to the same species.

Phylogenetic analyses by Relman et al., which included human-associated *Cyclospora* and three *Eimeria* species (two avian and one mammalian), supported the conclusion that *Cyclospora* and *Eimeria* belong to the same family of coccidian parasites (5). However, the authors noted that this apparent relatedness should be reevaluated when molecular data became available for additional *Eimeria* species and for *Isospora belli,* another coccidian parasite that causes diarrheal disease in humans.

Recently, the complete sequences of the small subunit ribosomal RNA (SSU-rRNA) gene of isolates of seven additional *Eimeria* species (six avian and one mammalian) were submitted to GenBank (6). We used these sequences, in addition to those previously available for human-associated *Cyclospora* and several *Eimeria* species (5), to reevaluate the phylogenetic relatedness of *Cyclospora* and *Eimeria* . Both of the previous phylogenetic analyses included sequences for *E. tenella* and *E. mitis* isolates; thus, we used two sequences for each of these species.

The structurally aligned sequences were retrieved from the Antwerp rRNA database (7); Mitchell L. Sogin kindly provided the alignment of the sequences used for the original molecular classification of *Cyclospora* (5). In addition to these two alignments, sequences were aligned with the ClustalW program (8). The aligned sequences were subjected to phylogenetic analysis with DNAPARS, NEIGHBOR, and DNAML programs from the PHYLIP package (9), using *Cryptosporidium parvum* (a coccidian parasite) or *Oxytricha granulifera* (a ciliate) as outgroups (all alignments are available from the authors upon request).

The topology of the phylogenetic tree obtained with all three methods was equivalent for all alignments. The maximum likelihood tree generated by the DNAML program from an alignment based on Sogin's approach is shown in the Figure. The topology of this tree, which includes human-associated *Cyclospora* and 12 isolates of 10 *Eimeria* species, is similar to that of the trees reported previously for *Cyclospora* and three species of *Eimeria* (5) and for nine species of *Eimeria* (6). The *Cyclospora* branch on the tree is between the branches of the eight avian and two mammalian *Eimeria* species that have been evaluated to date.

The results of the phylogenetic analysis strongly suggest that *Cyclospora* should be considered a member of the genus *Eimeria* , which is particularly noteworthy, since no organism

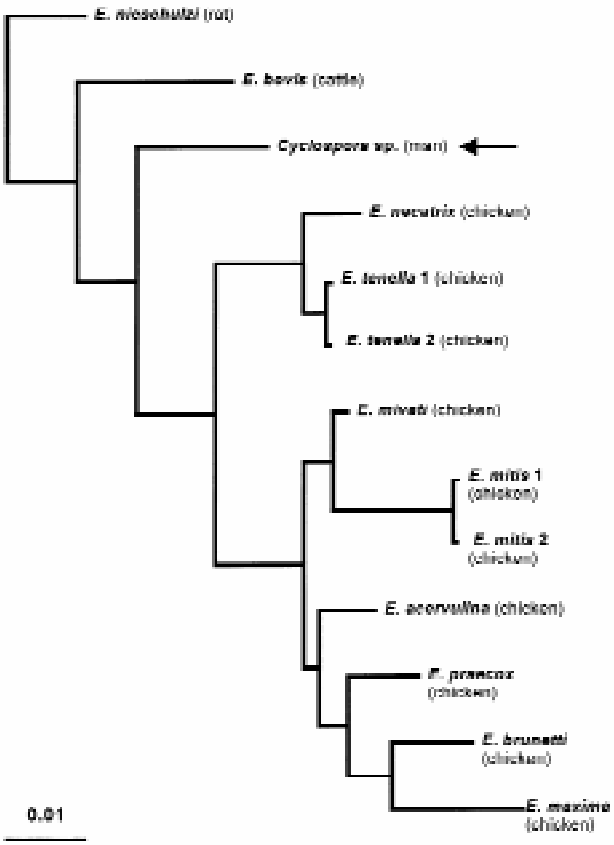

Figure. Phylogenetic tree for small subunit ribosomal RNA (SSU-rRNA) sequences of *Cyclospora* (marked by an arrow) and 12 isolates of 10 *Eimeria* species. Maximum likelihood analysis results using *Cryptosporidium parvum* as an outgroup are shown (ln likelihood = -5,421.96594). After analysis, the outgroup branch was removed to improve the readability of the tree. GenBank accession numbers for the sequences: human-associated *Cyclospora* sp. – U40261, *C. parvum* – L16996, *E. acervulina* – U67115, *E. bovis* – U77084, *E. brunetti* – U67116, *E. maxima* – U67117, *E. mitis* 1 – U67118, *E. mitis* 2 – U40262, *E. mivati* – U76748, *E. necatrix* – U67119, *E. nieschulzi* – U40263, *E. praecox* – U67120, *E. tenella* 1 – U67121, *E. tenella* 2 – U40264. Scale bar indicates an evolutionary distance of 0.01 nucleotides per position in the sequence.

currently classified as an *Eimeria* species is known to be pathogenic for humans. *Eimeria* is the largest genus of coccidian parasites and reportedly includes more than 1,500 named species (10). However, the current criteria (11) for naming "new" species of *Eimeria* (e.g., host specificity, morphologic characteristics of oocysts, duration of prepatent and patent periods, location of the infection in the host, and pathogenicity) are suboptimal, and the available data for some *Eimeria* species named in the past are incomplete. Thus, some *Eimeria* species may be synonymous, and some organisms thought to belong to the same species may not. The possibility even exists that human-associated *Cyclospora* is synonymous with a previously named *Eimeria* species. No molecular data are available for the type species of the *Cyclospora* genus or for the *Cyclospora* species that are not known to be human-associated. Reclassification, on the basis of phylogenetic analysis, of human-associated *Cyclospora* as an *Eimeria* species may stimulate productive research by suggesting possible animal reservoirs of human-associated *Cyclospora* (which may or may not infect other animals). In addition, animal models and cell culture systems that have been developed for *Eimeria* may prove useful for *Cyclospora* . However, it remains to be seen whether the biologic characteristics of *Cyclospora* are similar to those of the *Eimeria* species to which *Cyclospora* is closely related on the basis of phylogenetic criteria.

We also have preliminary data indicating that *I. belli* and human-associated *Cyclospora* do not belong to the same genus or family. *I. belli* oocysts (kindly provided by Alison Grant of Project RETRO-CI in Abidjan, Côte d'Ivoire) were gradient-purified, *I. belli* -specific DNA was extracted, and the SSU-rRNA gene was polymerase chain reaction-amplified and sequenced (Pieniazek et al., unpub. data). Sequence similarity searches of GenBank and preliminary phylogenetic analysis indicate that *I. belli* shares a more inclusive clade with members of the family Sarcocystidae than with the Eimeriidae (data not shown).

Molecular methods are arguably the best techniques available for studying the relatedness among organisms (11). To avoid confusion, reports of identification of *Cyclospora* (*Eimeria*) in animal hosts or in the environment should be supported by molecular data. Reports based on morphologic features alone (12-14) may suffer from poor resolution of features needed for classification of closely related organisms. To improve our understanding of the taxonomy of human-associated *Cyclospora* , molecular evaluation of isolates of additional *Cyclospora* and *Eimeria* species, especially other mammalian species, is needed.

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