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Phylogenetic Approach to the Variability of the Microsporidian *Enterocytozoon bieneusi* and Its Implications for Inter- and Intrahost Transmission[▽]

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***Enterocytozoon bieneusi* is a microsporidian parasite that infects many vertebrate animals, including humans. The rDNA internal transcribed spacer (ITS) shows a hypervariable sequence; however, so far no clear information has been inferred about strain evolution in this species. We reviewed all the sequences described and performed a phylogenetic study. Four groups of sequences strongly differentiated from each other were detected, although most of the isolates (94%) corresponded to group I. The highly diverse sequences of this group were analyzed using median-joining networks. The host species (humans, pets, swine, cattle, birds, and wild animals) and the continents of origin of the isolates were considered. Central haplotypes in the network were obtained from very diverse hosts and geographical origins. The results show that although *E. bieneusi* has a broad host specificity, transmission is not completely free: some strains were able to circulate within a given host species and were only occasionally transmitted to another host. Additionally, while not relevant for swine or cattle hosts, geography seems to be a relevant factor for human infection by *E. bieneusi*.**

Strict parasitism involves a high degree of adaptation to the host physiology. Often, parasites have lost a number of functions, and both the genome size and the number of genes may be decidedly reduced (28, 29). Microsporidia are intracellular parasites that infect all animal phyla (18). The infection occurs after a sophisticated interaction with the host cell (31, 65). These organisms are unicellular eukaryotes (included in the group of Fungi) that have lost mitochondria and have an extremely reduced genome (*Encephalitozoon cuniculi* has about 3 Mbp), although it is organized in several eukaryotic chromosomes (2, 30, 62). Some microsporidia seem to be host specific (9, 17), while others, such as the species of the genera *Encephalitozoon* and *Enterocytozoon*, may proliferate in various vertebrate species (4). Microsporidia of these two genera are usually considered opportunistic parasites, particularly in immunosuppressed individuals, such as AIDS patients (11, 12, 16). However, an increasing number of reports indicate that they are also frequent in immunocompetent people and animals (20, 38, 40).

From a clinical point of view, it is important not only to diagnose a given parasite correctly but also to differentiate among strains, since the similarities may evidence common sources of infection, and such information is necessary in order to design effective prophylactic measures for susceptible population groups. Unfortunately, microsporidia present some difficulties for molecular analysis. First, while *Encephalitozoon cuniculi*, *Encephalitozoon hellem*, and *Encephalitozoon intestinalis* may be cultured *in vitro* in different mammalian cell lines, *Enterocytozoon bieneusi* has thus far been impossible to culture (63), and DNA has to be extracted directly from clinical or environmental isolates (26, 39, 56). The internal transcribed spacer (ITS) of the ribosomal DNA (rDNA), located between the small-subunit and large-subunit ribosomal genes, has been widely used in the genotypic characterization of human microsporidia and has proved to be useful in rendering a reasonable variability in *E. cuniculi* and *E. hellem* (19, 25). In contrast, in the case of *E. intestinalis*, no genetic variability has yet been observed. Other polymorphic genetic markers, such as the PTP (polar tube protein) gene and the intergenic spacers (IGS) IGS-TH and IGS-HZ, have also been used to characterize intraspecies variability in *E. hellem* (25), as has the spore wall protein (SWP) gene in *E. cuniculi* (64).

The ITS is the only known polymorphic marker in *E. bieneusi*, and it shows a very high degree of variability compared to those of other microsporidia. It is about 243 bp long (which is relatively large for a microsporidian, since the ITS of the *Encephalitozoon* species mentioned above ranges from 28 to 45 bp). *E. bieneusi* has been isolated from different hosts, including humans and domestic and wild animals, and new variants of the ITS fragment are frequently described (51). Thus, the comparison of such sequences should provide valuable information about the transmission and evolution of *E. bieneusi*. Surprisingly, however, that hypervariability has so far precluded any relevant genotype classification. Some authors have outlined phylogenetic approaches (6, 8, 14, 34, 55, 56), but tree topologies differ among studies, and the statistical support for most nodes is poor. No clear relationships with the host or the geographical origins of the isolates have been inferred. In fact, there are almost 200 GenBank accession numbers for the ITS of *E. bieneusi*, but the picture is no clearer than a general zoonotic potential demonstrated by Dengjel et al. (14).

The study of the phylogeny of *E. bieneusi* strains is nevertheless very important, because this species infects a number of vertebrate hosts, including humans, from virtually all parts of the world. Within- and between-host transmission and the hy-

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TABLE 1. Number of ITS sequences of *E. bieneusi* per host and continent

Host	No. of ITS sequences[a]					
	Europe	North America	South America	Africa	Asia	Total
Humans	17	3	16	22 + 2	33	83 + 2
Swine	16	9	0	1	7	31
Cattle	7	12	0	0	7	26
Pets	8 + 2	0	7 + 1	1	1 + 2	17 + 5
Birds	4	0	0	0	1	5
Wild animals	2 + 1	15 + 3	0	0	0	17 + 4
Total	48 + 3	39 + 3	22 + 1	24 + 2	47 + 2	168 + 11

[a] Single numbers correspond to group I sequences; sums indicate the number of group I sequences plus sequences from any of the other groups (II, III, and IV). The rows and columns do not add up to the totals, because some sequences have been reported in more than one host or more than one continent.

pothetical preference of a specific genotype for a given host species have relevant epidemiological consequences. However, given the nature of the variability, the methods for phylogenetic inference have to be applied appropriately and correctly. This work approaches the whole set of complete ITS sequences of the microsporidian *E. bieneusi*. We aim to define the phylogenetic relationships among genotypes, to relate the variability with the origins of the isolates, and to provide a view of their epidemiological consequences.

MATERIALS AND METHODS

rDNA ITS sequences of *E. bieneusi*. *Enterocytozoon bieneusi* has been reported in a number of different host species, but molecular analyses have been performed only during the past decade. DNA sequences are available only for isolates from a small number of hosts, particularly (i) humans, (ii) swine, (iii) cattle, (iv) pets, including dogs, cats, and guinea pigs, (v) a heterogeneous group of bird species, and (vi) wild animals, including raccoons, foxes, beavers, muskrats, one monkey, one kudu, and one llama. (Some caution has to be exercised with this concept of "wild," since the two latter species were analyzed in Europe, and we do not know if they carried *E. bieneusi* from their localities of origin or became infected in the zoo.) The origins of the isolates are also quite diverse, including hosts from various countries. For further analyses, we will consider here only the continent of origin of the isolate: Europe, North America, South America, Africa, or Asia. A total of 179 complete sequences of the ITS of *E. bieneusi*, whose GenBank accession numbers and origins are given in the Appendix (a summary appears in Table 1), were analyzed in this study. A number of additional accession numbers do not cover the entire ITS (as mentioned previously, *E. bieneusi* cannot be cultured *in vitro* and has to be analyzed directly from the biological sample, so the amount and quality of the DNA may preclude optimal results) and were not included in this study, although they may provide important information about specific *E. bieneusi* outbreaks.

Methods for sequence analysis. The sequences given in the Appendix were obtained in FASTA format from GenBank and were aligned with Bioedit, version 7.0.5.2 (24), by following the Clustal W algorithm (60). Since the sequences differ in size because some include a variable fragment of one of the rRNA genes, the unequal tails at both sides were eliminated, and only the ITS fragment was considered. The haplotypes were generated with the DNAsp program, version 4.10 (47), without considering the occasional gaps. Haplotype diversity (Hd), the average number of nucleotide differences (k), nucleotide diversity (π), and Tajima's D (T's D; neutrality test) were also calculated with DNAsp. Neighbor-joining (NJ), UPGMA (unweighted-pair group method with average linkages), and minimum-evolution (ME) trees were constructed with the MEGA program, version 4.0 (58), using the Kimura 2-parameter (2P) model; maximum-likelihood (ML) trees were estimated with the Tree-Puzzle program, version 5.2 (53), and Bayesian trees with MrBayes, version 3.1.2 (27). Phylogenetic trees were edited with TreeView, version 1.6.6 (43). Haplotype median networks (3) were constructed with the Network program, version 4.5.1.0 (Fluxus Technology Ltd.). The Arlequin program, version 3.11 (22), was employed to estimate population differentiation; the fixation index (F_{st}) significance was estimated by a permutation test (n, 1,023 random permuted samples).

Nucleotide sequence accession numbers. The GenBank accession numbers of the 179 complete sequences of the ITS of *E. bieneusi* analyzed in this study are given in the Appendix.

RESULTS AND DISCUSSION

Differentiated groups of sequences. The alignment of the 179 sequences of the ITS region of *E. bieneusi* reveals four groups clearly divergent from each other. The same clades are obtained after the application of different methods for phylogenetic inference; the coincident maximum-likelihood and Bayesian unrooted tree is shown in Fig. 1. Group I includes most sequences (n, 168) and is analyzed below. Group II includes three sequences obtained from two human isolates from Africa and one marmoset analyzed in Portugal; in a total of 242 bp, only three polymorphic sites seem to exist (235A/G, 241A/G, and 242C/G). Group III corresponds to three sequences obtained from raccoon hosts in the United States; with a total length of 241 bp, there are some point differences (30C/G, 51T/−, 90−/G, 172G/C, and 229 G/A). Finally, the five sequences that make up group IV were all found in isolates from dogs, and one was also found in a cat, although the isolates were from different regions (Europe, South America, and Asia). The sequences constituting groups II, III, and IV did not correspond to specific outbreaks; instead, they appeared together with others analyzed in the same studies. For instance, the three sequences of group III were all obtained

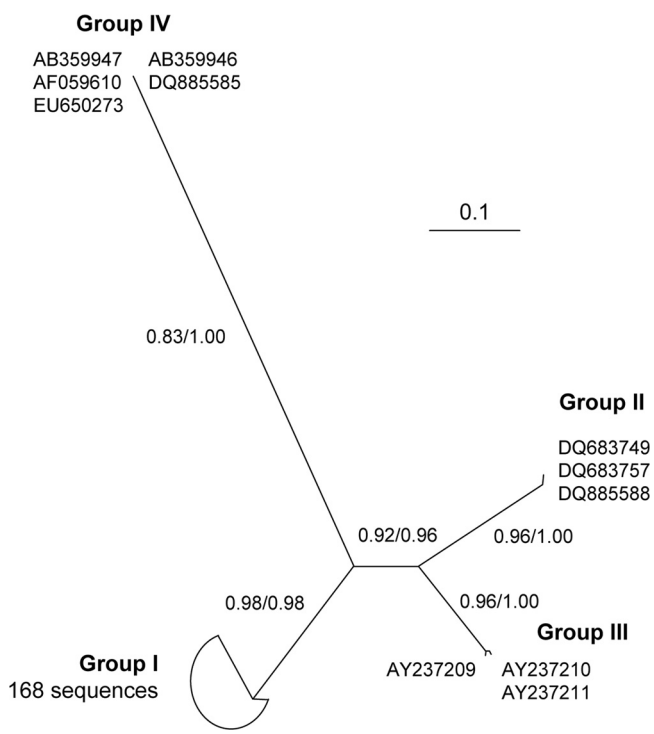

FIG. 1. Maximum-likelihood tree showing the four main groups of sequences, identified by the corresponding GenBank accession numbers. Group I comprises 168 sequences, all of which are in the enclosed area (bottom-left). The ML probability, followed by a slash and the posterior probability, is given on each branch. The bar represents 0.1 substitution per site.

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TABLE 2. Haplotype diversity, nucleotide diversity,[a] and Tajima's D for all group I sequences together and by host and continent

Category	No. of sequences	No. of haplotypes	Hd (SD)	k	π (SD)	Tajima's D	P^b
All	168	84	0.966 (0.007)	6.5	0.031 (0.002)	−1.809	<0.05
Host							
Humans	82	44	0.909 (0.028)	4.3	0.020 (0.002)	−2.033	<0.05
Swine	31	18	0.948 (0.021)	5.3	0.023 (0.002)	0.025	NS
Cattle	26	17	0.957 (0.023)	9.5	0.039 (0.006)	−0.800	NS
Pets	16	11	0.908 (0.063)	5.2	0.022 (0.008)	−1.967	<0.05
Wild animals	17	13	0.971 (0.028)	8.1	0.033 (0.007)	−0.246	NS
Birds	6	5	0.933 (0.122)	5.8	0.024 (0.008)	−0.722	NS
Continent							
Europe	49	32	0.977 (0.009)	7.6	0.033 (0.003)	−1.203	NS
North America	38	30	0.984 (0.011)	9.0	0.043 (0.005)	−1.294	NS
South America	23	16	0.964 (0.022)	3.9	0.016 (0.003)	−1.572	NS
Africa	24	15	0.949 (0.026)	2.7	0.011 (0.001)	−0.553	NS
Asia	47	23	0.817 (0.057)	5.0	0.023 (0.003)	−1.376	NS

[a] Hd, haplotype diversity; k, average number of nucleotide differences; π, nucleotide diversity.
[b] NS, not significant.

from raccoons by Sulaiman et al. (55), but these authors also found several sequences from group I in other specimens of the same host species. Group IV is even more suggestive, since exactly the same sequence was obtained from dogs in Europe, South America, and Asia by different authors, and again, each finding was accompanied by group I sequences.

It would be interesting to discover if the process of infection by the strains of groups II, III, and IV could be physiologically distinguished from that of typical group I *E. bieneusi* infections. Obviously, the concept of species within these microsporidia, for which sexual reproduction has never been described, is far from accurate; nevertheless, it is clear that within *E. bieneusi*, there are four groups of sequences strongly segregated from each other, with no intermediate forms. New findings might support a thorough analysis to clarify if the extent of divergence and hypothetical physiological differences are enough to confer a new taxonomic status on these groups.

Diversity within group I. Group I comprises 94% (168/179) of the sequences so far detected for the ITS of *E. bieneusi*. The sequence obtained from an isolate from *Macaca mulatta* (GenBank accession no. AF023245) (10) was used as a reference. This group exhibits high diversity, mainly due to single nucleotide polymorphisms (SNP), with occasional insertion or deletion events of single base pairs; longer indels have not been detected. A total of 74 polymorphic sites (excluding gaps) exist along the whole 243-bp length of the ITS. Accordingly, the nucleotide diversity (π) was high, 0.0311, and the average number of nucleotide differences (k) was 6.5.

The 168 sequences can be classified into 84 different haplotypes (see the Appendix), which match with the groups established by Santín and Fayer (50), except for sequences AF267147, DQ885580, and DQ425108, which were included in haplotypes 10, 13, and 21 (hap-10, -13, and -21), respectively, because after the positions with gaps were removed, a coincident SNP could not be considered, either. The haplotypes are unevenly distributed among the isolates. Thus, 54 haplotypes (64%) were unique findings, while others were found repeatedly in a single host. For instance, hap-10 was obtained from human isolates by different authors, and hap-57 was obtained repeatedly from cattle. In contrast, many haplotypes can be obtained from very diverse origins: hap-1, hap-11, and hap-13 have been reported in different studies from all the hosts surveyed (humans, swine, cattle, wild animals, pets, and/or birds) from practically everywhere.

Haplotype and diversity indices for group I sequences were high within individual host species and within continents.

Phylogenetic relationships. After the comparison of the first 20 sequences described for the ITS of *E. bieneusi*, Dengjel et al. (14) observed that there were no clades specific for a given host, which appeared mixed in the phylogenetic trees (ML, NJ, and maximum-parsimony [MP] trees were analyzed)—i.e., the same host appeared in different clades, and a given clade may include genotypes from different hosts. Those authors predicted that it was a matter of time until identical sequences would be obtained from humans and animals. Haplotypes common to different host species have indeed been confirmed by several authors (see the Appendix), supporting the zoonotic nature of *E. bieneusi* (34), and at least some genotypes have broad host specificity (55, 56). However, except for this general idea, previous studies could not establish whether the genotypes are actually completely mixed—and hence *E. bieneusi* could perhaps circulate freely among the different host species—or whether some genotypes are restricted to a given host. In an NJ tree of 68 sequences, Cama et al. (8) noted a group of so-called "animal genotypes" and another of "genotypes reported in humans and animals." Most branches in the phylogenetic trees are very short, and except for identical sequences, groups are ill-defined, the statistical significance of most nodes is poor, and the final topology may depend strongly on slight changes in the method used for phylogenetic inference. A possible influence of the geographical origin of the *E. bieneusi* isolates has never been investigated.

Group I sequences have to be analyzed independently, because the other groups (II, III, and IV) are clearly segregated and diverge strongly from all the group I sequences. Although the diversity within group I is high, the differences between any pair of sequences are due to a small number of point differences (Table 2). Additionally, we have to keep in mind that the

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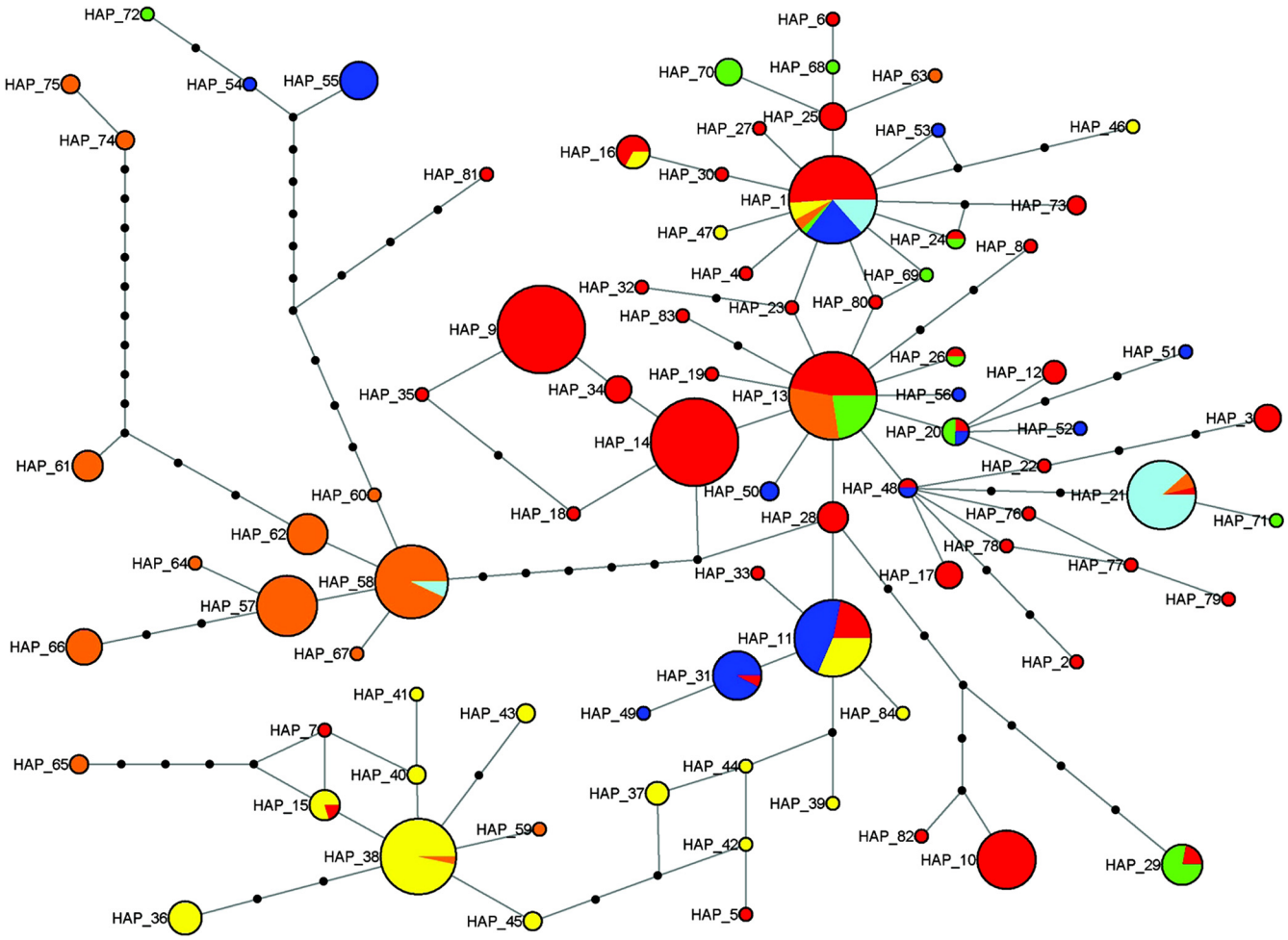

FIG. 2. Median-joining network for the 84 haplotypes of the group I sequences. Circles are proportional to the frequency of each haplotype. The color of each sector represents the host species: humans (red), swine (yellow), cattle (orange), pets (green), birds (light blue), and wild animals (dark blue). Each step on the branches represents a single mutation.

ancestral haplotypes may persist in the populations and that a given haplotype may produce several derivatives. Thus, for such intraspecific variability with low divergence, a phylogenetic network is a better option than a tree. Median-joining networks (3) were constructed with the Network program. According to the first run of calculations, one base pair (nucleotide 30 in the ITS sequence) was involved in 26 mutational events, while the frequencies for any other changes were all less than or equal to 5, so the weight of that point was lowered to 5/10 before the calculations were repeated. The frequency of each haplotype was also considered in the final graph (Fig. 2), although these frequencies should be considered with caution, because some reports, especially for those sequences referenced only in GenBank, do not specify the number of isolates in which a given sequence was found (we assumed the minimum number was 1).

Most haplotypes can be related to another by a low number of mutations, thus forming a tight network. However, some haplotypes or haplotype groups are weakly attached to the main body of the network (hap-61, hap-74 and -75, hap-55, -54, and -72, hap-10 and -82, and hap-29). As theoretically expected, central haplotypes—particularly hap-1 and hap-13–were obtained from more diverse hosts and geographical origins, and they also had more "satellites." Presumably, they represent the most ancient ITS haplotypes of *E. bieneusi*. The distribution of the different groups of hosts along the complete network is certainly not segregated, but apparently, neither are hosts randomly distributed. Two clades segregate from the main body of the network; one is composed of haplotypes mostly obtained from cattle isolates and the other of swine

TABLE 3. F_{st} values between pairs of host populations, calculated with the Arlequin program using the Kimura 2P distance method

Host	F_{st}[a]				
	Humans	Swine	Cattle	Pets	Wild animals
Swine	0.287***				
Cattle	0.272***	0.278***			
Pets	0.018	0.261***	0.211***		
Wild animals	0.096***	0.221***	0.162***	0.025	
Birds	0.097	0.262**	0.049	0.021	0.044

[a] Significance was estimated after the permutation test (n = 1,023). **, $P < 0.01$; ***, $P < 0.001$.

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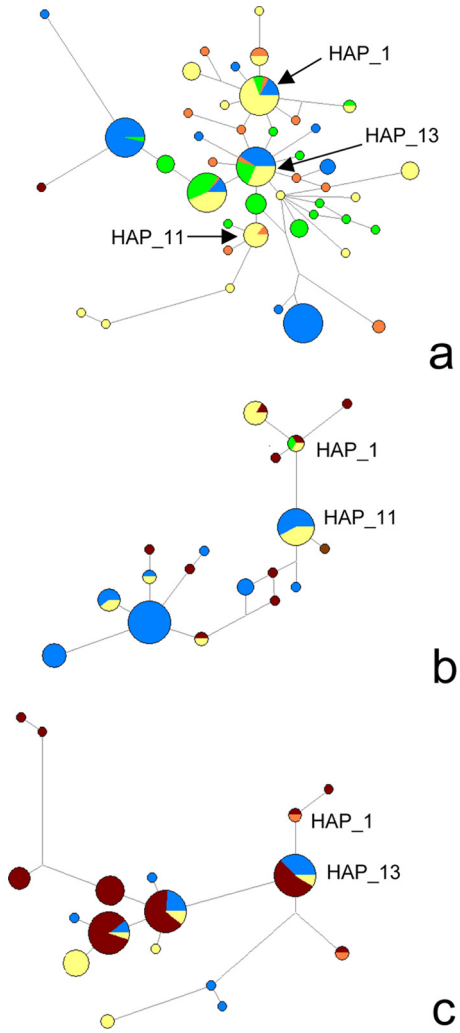

FIG. 3. Median-joining networks for group I haplotypes found in humans (a), swine (b), and cattle (c). Circles are proportional to the frequency of each haplotype. The colors refer to the continents where the isolates were found: Europe (blue), North America (dark brown), South America (orange), Africa (green), and Asia (yellow). The positions of the haplotypes on the graphs are approximately the same as in Fig. 2; for reference, hap-1, hap-11, and hap-13 are indicated. The lengths of the segments are proportional to the number of mutations involved between two haplotypes.

haplotypes. The haplotypes from wild animals showed high diversity (Table 2) and were indeed distributed all along the network (Fig. 2). This fact is even more remarkable considering that studies of microsporidia of wild fauna are very scarce (for instance, haplotypes 1, 11, 31, 49, 51, 54, and 55 were all obtained from muskrats by Sulaiman et al. [55]), so we may conjecture that any new ITS haplotype obtained from a wild animal can attach anywhere on the network.

Population differentiation. (i) Among hosts. As shown in Fig. 2, the haplotypes obtained from humans, pets, and wild animals are apparently distributed throughout the network. According to the F_{st}, calculated with the Arlequin program by using the Kimura 2P distance method and given in Table 3, no barriers appear to exist between human and pet isolates. The wild-animal population shows a significant F_{st} compared to human isolates but not compared to pet isolates. In contrast, the populations of swine and cattle isolates are deeply differentiated, with high F_{st} values. For each of these two hosts, there is a group of related haplotypes. However, these groups are not exclusive and include occasional isolates from other hosts: a chicken isolate in the cattle group (hap-58) and, in the swine group, isolates of human (hap-5, -7, and -15) or cattle (hap-59 and -65) origin. Furthermore, some cattle or swine isolates also match haplotypes on other parts of the network (hap-1, -16, -46, and -47 for swine and hap-13, -21, and -63 for cattle).

Tajima's D (Table 2) showed significant negative values for the total population of group I sequences and also for those of human and pet isolates (if these two are pooled, T's D is still significant). Negative values indicate a high divergence in the terminal branches of the phylogenetic trees, usually due to positive selection or to population expansion (57). As we have shown above, some groups of haplotypes tend to exist in a given host species, particularly cattle (hap-57, hap-58 and satellites) or swine (hap-38 and satellites), and it is also clear that *E. bieneusi* may be transmitted from one host species to another. Thus, a haplotype exhibiting important differences from the common haplotypes in a given host may be explained, not by several mutations, but simply by infection with strains usually circulating in another host. Haplotypes 5 and 15 may be good examples: if we built a network using only the haplotypes of human origin (Fig. 3a), hap-15 and hap-7 are distant from the torso and hence could be interpreted as diverging strains, but once the other hosts are included, the presence of these haplotypes in human patients is easily explained by a swine origin of the strains. The same could be inferred for pets: hap-73 is far from any other dog or cat isolate, but it is

TABLE 4. F_{st} values between pairs of continents within hosts, calculated with the Arlequin program using the Kimura 2P distance method

Continent[a]	F_{st}[b]							
	Humans				Swine		Cattle	
	Europe	N. America	S. America	Africa	Europe	N. America	Europe	N. America
N. America	0.298*				0.003		0.131*	
S. America	0.096*	0.341**			—[c]	—	—	—
Africa	0.121**	0.473***	0.035		—	—	—	—
Asia	0.135***	0.387**	0.079*	0.043*	−0.049	−0.109	−0.047	0.032

[a] N., North; S., South.
[b] Significance was estimated after the permutation test (n = 1,023). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[c] —, comparison not performed.

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similar to hap-54 and -55, suggesting infection with a wild-animal isolate.

Nevertheless, the present set of hosts does not seem to explain the whole variability, since T's D is still significantly negative (Table 2), with some haplotypes weakly linked to the network (Fig. 2). One possible explanation is that divergent haplotypes are actually the product of a hypothetical directional selection, at present of unknown origin (obviously not directly on the ITS, which is a noncoding sequence, but it may record directional selection on other loci by genetic hitchhiking). However, it is important to remember that *E. bieneusi* is a parasite with a wide range of vertebrate hosts, and the isolates analyzed from a molecular standpoint correspond only to the host species already mentioned. For instance, rabbits could be regular hosts (13), but no ITS sequences are available from that source yet. It is possible that the study of isolates from new host species can fill those gaps.

(ii) Among continents. The comparison of continents can be affected by the asymmetric distribution of the hosts analyzed so far; for instance, most wild-animal isolates were from North America, where no isolates from pets or birds have been described (Table 1). Therefore, we have analyzed the within-host continent variation only for humans, swine, and cattle, for which several isolates exist from different regions (Europe, North America, and Asia). The F_{st} between pairs of populations are shown in Table 4. The human results contrast strongly with those for swine and cattle. For humans, most F_{st} are significant, and only South America and Africa are not significantly differentiated from each other; the North American population of *E. bieneusi* isolates showed the strongest isolation from the remainder. In contrast, the populations of isolates from swine are not segregated, since no F_{st} are statistically different from zero. A similar picture emerges for cattle isolates, although the F_{st} between Europe and North America was only slightly significant (P, 0.05). The distributions among continents of the haplotypes found in both swine and cattle (Fig. 3b and c) do not indicate recent colonization in these species, either. Thus, the circulation among swine or cattle seems to be quite free, while the transmission of *E. bieneusi* in humans is clearly influenced by geography.

Concluding remarks. The high variability of the ITS fragment allows a general picture of the transmission of *E. bieneusi* both among host species and within a given host. First, occasional sequences (groups II, III, and IV) strongly divergent from the "classic" genotypes (group I) have been obtained from different origins. Further studies should determine the extent of divergence and a possible relationship with the clinical features of *E. bieneusi* infection. We agree with Santín and Fayer (50) that new sets of markers, independent of the ITS, would be necessary to support the epidemiology of this species. Second, as several authors have indicated, *E. bieneusi* has a broad host specificity, but we have shown that transmission is not completely free. Humans, cats, and dogs are interdependent, and the diversity of haplotypes suggests an ancient infection from different sources, including human-to-human transmission. In contrast, the segregation of cattle and swine hosts suggests a certain degree of host specificity: some genotypes would circulate freely within a given host but also may occasionally be transmitted to another host. This would explain why some haplotypes are so divergent from those more common to the same host. Screening for *E. bieneusi* haplotypes in other hosts, particularly wild animals, may find some "missing links" between the main network of sequences and those significantly detached.

APPENDIX

Table A1 shows the classification by group and haplotype of the 179 complete rDNA ITS sequences of *E. bieneusi* analyzed in this study, along with their GenBank accession numbers and origins.

TABLE A1. Classification by group and haplotype of the complete rDNA ITS sequences of *E. bieneusi* analyzed in this study

Group	GenBank accession no.	Genotype[a]	Haplotype	No. of isolates with the ITS sequence												Reference(s)
				All	By host						By continent[b]					
					Humans	Swine	Cattle	Pets	Wild animals	Birds	Eu	NA	SA	Af	As	
I	AB470282		hap-1	1		1									1	GB[c]
I	AF023245	D	hap-1	1					1			1				10
I	AF101200	D	hap-1	18	18						4			1	13	21, 34, 45, 48
I	AF348477	D	hap-1	1		1						1				7
I	AY237216	D	hap-1	9					9			9				54
I	AY371284	D	hap-1	2	1		1					1	1			51, 55
I	DQ683751	D	hap-1	1	1									1		6
I	DQ683755	D	hap-1	3	3									3		6
I	DQ793213	D	hap-1	6						6					6	42
I	DQ885582	D	hap-1	1				1			1					36
I	EF139197	D	hap-1	1			1								1	32
I	FJ469916		hap-1	1		1								1		GB
I	AY945808	R	hap-2	1	1										1	34
I	AY945809	S	hap-3	4	4										4	34
I	AY945810	T	hap-4	1	1										1	34
I	AY945811	U	hap-5	1	1										1	34
I	AY945812	V	hap-6	1	1										1	34
I	AY945813	W	hap-7	1	1										1	34
I	AF242479	Type V	hap-8	1	1						1					35

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TABLE A1—*Continued*

Group	GenBank accession no.	Genotype[a]	Haplotype	No. of isolates with the ITS sequence												Reference(s)
				All	By host						By continent[b]					
					Humans	Swine	Cattle	Pets	Wild animals	Birds	Eu	NA	SA	Af	As	
I	AF101198	B	hap-9	23	23						23					5, 45, 48
I	AF242475	B	hap-9	66	66						66					35
I	DQ683754	B	hap-9	3	3									3		6
I	AF101199	C	hap-10	9	9						9					5, 14, 45
I	AF242476	C	hap-10	9	9						9					35
I	AF267147	Q	hap-10	1	1						1					14
I	AB470284		hap-11	1		1									1	GB
I	AF076042	EbpC	hap-11	7		7					7					5
I	AF135832	EbpC	hap-11	7	6	1					1				6	15, 21, 34
I	AY237221	EbpC	hap-11	13					13			13				54
I	AY237225	EbpC	hap-11	2					2			2				54
I	AY371279	EbpC	hap-11	1	1								1			55
I	U61180 s	EbpC	hap-11	1		1					1					15
I	AF242477	Type III	hap-12	3	3						3					35
I	AB359945		hap-13	1				1							1	1
I	AF242478	Type IV	hap-13	11	9		2				9				2	32, 35
I	AF267141	Type IV	hap-13	10	8			2			3			7		14, 21, 48, 56
I	AY331009	Type IV	hap-13	12			12					12				51, 56
I	AY331010	Type IV	hap-13	1			1					1				GB
I	AY371277	Type IV	hap-13	1	1								1			55
I	DQ683752	Type IV	hap-13	4	4									4		6
I	DQ683756	Type IV	hap-13	1	1									1		6
I	DQ836343	Type IV	hap-13	2				2					2			52
I	DQ885579	Type IV	hap-13	4				4			4					36
I	DQ885580	PtEb IV	hap-13	1				1			1					36
I	EU650272	Type IV	hap-13	1				1					1			49
I	AF101197	A	hap-14	16	16						6			10		5, 21, 45
I	AY357185 to -204	A	hap-14	21	21										21	33
I	AY371276	A	hap-14	1	1								1			55
I	DQ683750	A	hap-14	1	1									1		6
I	DQ683753	A	hap-14	8	8									8		6
I	AF267145	O	hap-15	3	1	2					1				2	14, 32, 34
I	EU849127		hap-15	1		1					1					GB
I	EU849128		hap-15	1		1					1					GB
I	AF348475	PigEBITS7	hap-16	6	4	2						1			5	6, 32, 34
I	AF502396	UG2145	hap-17	4	4									4		61
I	AY315716		hap-18	1	1							1				33
I	AY371278	Peru3	hap-19	1	1								1			55
I	AY237219	WL11	hap-20	1					1			1				54
I	AY371280	WL11	hap-20	1	1								1			55
I	DQ836344	WL11	hap-20	1				1					1			52
I	EU650271	WL11	hap-20	1				1					1			49
I	AY371281	Peru6	hap-21	23	1		1			21	21	1	1			37, 51, 55
I	DQ154137	Peru6	hap-21	1			1					1				51
I	DQ425107	Peru6	hap-21	1						1	1					37
I	DQ425108	PtEb II	hap-21	1						1	1					37
I	AY371282	Peru7	hap-22	1	1								1			55
I	AY371283	Peru8	hap-23	1	1								1			55
I	AY371285	Peru10	hap-24	1	1								1			55
I	DQ836342	Peru10	hap-24	1				1					1			52
I	AY371286	Peru11	hap-25	3	3								1		2	34, 55
I	EF014428	Peru11	hap-25	1	1								1			55
I	DQ683747	CAF2	hap-26	1	1									1		6
I	FJ469915		hap-26	1				1						1		GB
I	DQ683748	CAF3	hap-27	1	1									1		6
I	DQ683746	CAF1	hap-28	5	5									5		6, 21
I	EF014427	Peru16	hap-29	9	2			7					9			8, 55
I	EF014429	Peru13	hap-30	1	1								1			55
I	AY237223	WL15	hap-31	7					7			7				54
I	AY237224	WL15	hap-31	5					5			5				54
I	EF014430	WL15	hap-31	1	1								1			55
I	EF014431	Peru15	hap-32	1	1								1			55
I	EF458627	HAN1	hap-33	1	1										1	21
I	EF458628	NIA1	hap-34	3	3									3		21
I	FJ469914		hap-34	1	1									1		GB
I	L20290 h		hap-35	1	1							1				66
I	AF076041	EbpB	hap-36	6		6					6					5
I	AF076043	EbpD	hap-37	3		3					3					5
I	AB470279		hap-38	1		1									1	GB
I	AF076040	EbpA	hap-38	12		12					12					5
I	AF135833	EbpA	hap-38	16		15	1				16					14, 46
I	EU849130		hap-38	1		1					1					GB
I	EU849132		hap-38	1		1					1					GB
I	AF135834	G	hap-39	1		1					1					46

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TABLE A1—*Continued*

Group	GenBank accession no.	Genotype[a]	Haplotype	No. of isolates with the ITS sequence												Reference(s)
				All	By host						By continent[b]					
					Humans	Swine	Cattle	Pets	Wild animals	Birds	Eu	NA	SA	Af	As	
I	AB470280		hap-40	1		1									1	GB
I	AF135835	H	hap-40	1		1					1					46
I	AF348469	PigEBITS1	hap-41	1		1						1				7
I	AF348470	PigEBITS2	hap-42	1		1						1				7
I	AF348471	PigEBITS3	hap-43	1		1						1				7
I	EU883783		hap-43	1		1					1					GB
I	AF348472	PigEBITS4	hap-44	1		1						1				7
I	AB470281		hap-45	1		1									1	GB
I	AF348473	PigEBITS5	hap-45	1		1						1				7
I	AF348474	PigEBITS6	hap-46	1		1						1				7
I	AF348476	PigEBITS8	hap-47	1		1						1				7
I	DQ885581	PtEb V	hap-48	1					1		1					36
I	FJ439681		hap-48	1	1									1		59
I	AY237222	WL14	hap-49	1					1			1				54
I	AY237220	WL12	hap-50	2					2			2				54
I	AY237218	WL10	hap-51	1					1			1				54
I	AY237217	WL9	hap-52	1					1			1				54
I	AY237215	WL7	hap-53	1					1			1				54
I	AY237214	WL6	hap-54	1					1			1				54
I	AY237212	WL4	hap-55	7					7			7				54
I	AY237213	WL4	hap-55	1					1			1				54
I	AF267146	P	hap-56	1					1		1					14
I	AF135836	I	hap-57	2			2				2					46
I	AY331006	I	hap-57	17			17					17				51, 56
I	EF139199	I	hap-57	1			1								1	32
I	AF135837	J	hap-58	6			4			2	6					14, 44, 46
I	AY331005	J	hap-58	19			19					19				51, 56
I	DQ885586	J	hap-58	1			1				1					36
I	EF139196	J	hap-58	3			3								3	32
I	AF267143	M	hap-59	1			1				1					14
I	AF267144	N	hap-60	1			1				1					14
I	AY331007	BEB3	hap-61	5			5					5				51, 56
I	AY331008	BEB4	hap-62	9			9					9				51, 56
I	DQ154136	4948FL2	hap-63	1			1					1				51
I	DQ885587	PtEb XI	hap-64	1			1				1					36
I	EF139198	CEbD	hap-65	2			2								2	32
I	EF139195	CEbA	hap-66	7			7								7	32
I	EF139194	CEbF	hap-67	1			1								1	32
I	AF118144		hap-68	1				1			1					41
I	AF267142	L	hap-69	1				1			1					14
I	DQ836345	D-like	hap-70	4				4					4			52
I	DQ885583	Peru6	hap-71	1				1			1					36
I	DQ885584	PtEb VIII	hap-72	1				1			1					36
I	AY168419 (r.c.)[d]		hap-73	1	1										1	GB
I	AY298730 (r.c.)		hap-73	1	1							1				GB
I	EU153584	BEB6	hap-74	1			1					1				23
I	EU153585	BEB7	hap-75	1			1					1				23
I	FJ439677		hap-76	1	1									1		59
I	FJ439678		hap-77	1	1									1		59
I	FJ439679		hap-78	1	1									1		59
I	FJ439680		hap-79	1	1									1		59
I	FJ439682		hap-80	1	1									1		59
I	FJ439683		hap-81	1	1						1					59
I	FJ439684		hap-82	1	1						1					59
I	FJ439685		hap-83	1	1						1					59
I	EU849129		hap-84	1		1					1					GB
II	DQ683749	CAF4		4	4									4		6
II	DQ683757	CAF4		5	5									5		6
II	DQ885588	PtEb XII		1					1		1					36
III	AY237209	WL1		4					4			4				54
III	AY237210	WL2		2					2			2				54
III	AY237211	WL3		1					1			1				54
IV	AF059610	PtEb IX		3				1			3					41
IV	DQ885585	PtEb IX		1				1			1					36
IV	EU650273	PtEb IX		1				1					1			49
IV	AB359946			1				1							1	1
IV	AB359947			1				1							1	1

[a] Primary genotype name according to the 2009 revision by Santín and Fayer (50).
[b] Eu: Europe; NA, North America; SA, South America; Af, Africa; As, Asia.
[c] GB, published only in GenBank.
[d] r.c., reverse complementary sequence.

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