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Enterocytozoon bieneusi Genotype Nomenclature Based on the Internal Transcribed Spacer Sequence: A Consensus

MÓNICA SANTÍN and RONALD FAYER

Environmental Microbial Safety Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705

ABSTRACT. The standard method for determining the genotypes of *Enterocytozoon bieneusi* is based on the DNA sequence of the internal transcribed spacer (ITS) region of the rRNA gene. There are 81 genotypes with 111 genotype names: 26 genotypes have been identified exclusively in humans, eight have been identified in humans and in other hosts, 27 have been identified exclusively in cattle and pigs, six have been identified exclusively in cats and dogs, and 14 have been identified in miscellaneous hosts. Because none of these genotypes has taxonomic status and therefore do not adhere to the International Code of Zoological Nomenclature regarding naming, some genotypes have received multiple names, each different and in separate publications by different authors. Because of the proliferation of genotypes with overlapping names and multiple hosts the scientific literature has become confusing and difficult to efficiently utilize. To reduce confusion and provide guidance for future publications we tabulated all names, GenBank accession numbers, and author citations and propose that the first published name has precedence and should become the primary name used in all subsequent publications in which genotyping is based on ITS sequencing. In those publications the names and GenBank numbers that were submitted at later dates should also be provided by the authors as synonyms to aid readers and reviewers.

Key Words. terminology, microsporidia, genotyping.

WITHIN the Microsporidia are 14 species infectious for humans (Didier and Weiss 2006). The most common species of Microsporidia reported to infect humans is *Enterocytozoon bieneusi*, the only species in the genus (Mathis, Weber, and Deplazes 2005). First found in the enterocytes of a patient with AIDS, *E. bieneusi* was described solely on morphologic features (Desportes et al. 1985). Over 20 years later this species is recognized as a ubiquitous enteric protist parasite with extensive genetic diversity. Based on molecular differences among *E. bieneusi* isolates from fecal specimens from humans, many domestic and wild mammals, and birds, many genotypes have been described (Breitenmoser et al. 1999; Lobo et al. 2006a, b; Reetz et al. 2002; Santín, Cortes Vecino, and Fayer 2008; Santín, Trout, and Fayer 2005; Santín et al. 2006; Sulaiman et al. 2003a, b). Most studies on human microsporidiosis have included HIV-infected patients with diarrhea, but recently, *E. bieneusi* infections have been detected in immunocompetent persons (Breton et al. 2007; Nkinin et al. 2007; Samie et al. 2007). Although infection with *E. bieneusi* has been linked to diarrhea, some hosts are clearly asymptomatic (Samie et al. 2007). Therefore, it is not clear if *E. bieneusi* is always a primary human pathogen. Viewing *E. bieneusi* as a complex consisting of numerous genotypes, each with unique biological attributes, may provide a perspective from which we can better assess the diversity of host specificity and pathogenicity.

Current typing of *E. bieneusi* relies on molecular methods because differences among *E. bieneusi* isolates cannot be discriminated morphologically. Sequencing of the internal transcribed spacer (ITS) region of the rRNA gene is the standard method for genotyping *E. bieneusi*. The ITS region has shown a high degree of diversity among isolates, and genotype names have been established using unique and partially overlapping nomenclatures. The varying terminologies that have been used to designate genotypes of *E. bieneusi* that were detected in a particular study make the comparison of published studies confusing and identification of new isolates difficult. Some genotypes have received as many as six different names, the names varying with authors (see genotype D in Table 2). This lack of an acceptable nomenclature for naming ITS sequences has resulted in multiple genotype designations being applied to the same ITS nucleotide sequence. Such great genetic diversity within *E. bieneusi* (based solely on ITS sequence differences) has contributed (i) to confusion regarding the role of *E. bieneusi* as a disease agent because it is especially difficult to conclude if genotypes differ in their effect on the host and (ii) to uncertainty regarding the zoonotic potential of genotypes reported in both human and animal fecal specimens.

At the Tenth International Workshop on Opportunistic Pathogens (IWOP-10), a roundtable discussion entitled ''Terminology for *Enterocytozoon bieneusi*'' was held with the aim of proposing a plan to reduce confusion associated with the identification of genotypes within *E. bieneusi*. The following persons who attended the roundtable have read and approved of the present report, and agreed to use the proposed method of identification for *E. bieneusi* when writing and reviewing manuscripts: Donna Akiyoshi, Ann Cali, Carmen del Águila, Vitaliano Cama, Elizabeth Didier, Ronald Fayer, Yaoyu Feng, Xiaochuan Feng, J. Russell Hayman, Edna Kaneshiro, Olga Matos, Mónica Santín, Abhinneet Sheoran, Julia Sokolova, Eric Villegas, Govinda Visvesvara, Louis Weiss, Giovanni Widmer, Lihua Xiao, and Quanshun Zhang.

Two plans were proposed. For the near future: a standardized nomenclature for *E. bieneusi* genotypes based on ITS sequences to facilitate investigations of the epidemiology and pathogenicity. This plan is proposed within this document. For the more distant future: inclusion of additional genetic markers to improve the resolution of *E. bieneusi* genotyping methods.

ATTEMPT AT AN EARLY PLAN BASED ON ITS SEQUENCES TO IDENTIFY HOST RELATIONS

A phylogenetic analysis was conducted on all *E. bieneusi* ITS sequences available in GenBank in 2005 (Drosten et al. 2005). This system proposed by Drosten et al. separated *E. bieneusi* into three major groups and an outlier group, all supported by bootstrap values higher than 80%. Three groups contained genotypes from only one host animal species suggesting host restriction of these groups. For example, group 2 contained only cattle, group 3 contained only muskrat, and group 4 (the outlier) contained only raccoon hosts. None was subdivided into clades. Group 1 contained multiple host species and some clades that appeared to be host restricted, associated only with humans or only with pigs. The zoonotic potential of *E. bieneusi* was phylogenetically supported by the diversity of host species within other clades in group 1, which included human, macaque, swine, cat, fox, and

Corresponding Author: Ronald Fayer, Environmental Microbial Safety Laboratory, Agricultural Research Service, United States Department of Agriculture, 10,300 Baltimore Avenue, Beltsville, MD 20705—Telephone number: +1 301 504 8750; FAX number: +1 301 504 6608; e-mail: ronald.fayer@ars.usda.gov

beaver hosts; or human, cattle, llama, cat, fox, beaver, and muskrat hosts; or human, swine, raccoon, and muskrat hosts. Unfortunately, sequences obtained from GenBank in 2008 fail to support this system because ITS sequences subsequently deposited in GenBank identified the presence of multiple host species in all proposed groups, rendering the groupings based on host specificity invalid.

THE PROPOSED PLAN FOR DESIGNATING *ENTEROCYTOZOON BIENEUSI* GENOTYPES BASED ON ITS SEQUENCING

Most of the primers reported in the literature for genotypig *E. bieneusi* have amplified the ITS region. Some have also included a portion of the flanking regions consisting of the large and small subunit ribosomal RNA gene. Within this article, all *E. bieneusi* ITS sequences available in GenBank as of June 1, 2008, have been summarized. In total, 165 *E. bieneusi* ITS sequences were obtained from GenBank. Fourteen ITS sequences excluded from the analysis because they were incomplete sequences include accession numbers: EF661812–EF661814, EF010879–EF010883, AY668952, AY668953, AY298730, AY315716, L20290, and AH012971.

Within Tables 1–5 are all the reported genotypes of *E. bieneusi*, including information about the host, genotype name(s), GenBank sequence numbers, and references. Where multiple names appear for the same ITS sequence, the first recorded name for that sequence is listed as the primary name with subsequent names listed as synonyms. Table 1 consists of genotypes found only in humans. Table 2 consists of zoonotic genotypes. Table 3 consists of genotypes found only in livestock such as pigs and cattle. Table 4 consists of genotypes found only in companion animals such as dogs and cats. Table 5 consists of genotypes found in miscellaneous hosts. A total of 81 genotypes were identified with 111 genotype names: 26 genotypes were identified exclusively in humans, eight were identified in humans and in other hosts, 27 were identified exclusively in cattle and pigs, six were identified exclusively in cats and dogs, and 14 were identified in miscellaneous hosts. Some of the genotypes with an identical ITS sequence were given different names based on nucleotide differences in regions flanking the ITS region, small and large subunits of the rRNA gene; for example, Peru11 and Peru12 (Table 1) or WL15 and WL16/Peru14 (Table 2).

The first published name has precedence and should become the primary name used in all subsequent publications in which genotyping information based on ITS sequencing is reported. In those subsequent publications, the names and GenBank numbers that were submitted at later dates should also be provided by the authors as synonyms to aid readers and reviewers. For example, genotype A reported in 1997 should be the primary name that takes precedence over genotype Peru1 published in 2003 and the latter name should be cited only as a synonym. Names for new isolates should be at the discretion of the authors and each new name should be clearly associated with a GenBank number. When submitting new nucleotide sequences to GenBank, the submitters should clearly indicate the host and synonymized

Table **1.** *Enterocytozoon bieneusi* genotypes identified by the internal transcribed spacer (ITS) sequence and reported only in humans.

Host	Primary genotype name (GenBank accession number)	Synonyms (GenBank accession number)	References
Humans	A (AF101197, AY168419[a], AY357185–AY357204, DQ683750, and DQ683753)	Peru1 (AY371276)	Breton et al. (2007), Leelayoova et al. (2005), Rinder, Katzwinkel-Wladarsch, and Loscher (1997), Sulaiman et al. (2003a)
	B (AF101198 and DQ683754)	Type I (AF242475)	Breton et al. (2007), Liguory et al. (1998), Rinder et al. (1997)
	C (AF101199)	Type II (AF242476)	Liguory et al. (1998), Rinder et al. (1997)
	Q (AF267147)[b]		Rinder et al. (2000)
	R (AY945808)		Leelayoova et al. (2006)
	S (AY945809)		Leelayoova et al. (2006)
	T (AY945810)		Leelayoova et al. (2006)
	U (AY945811)		Leelayoova et al. (2006)
	V (AY945812)		Leelayoova et al. (2006)
	W (AY945813)		Leelayoova et al. (2006)
	Type III (AF242477)		Liguory et al. (1998)
	Type V (AF242479)		Liguory et al. (1998)
	Peru3 (AY371278)		Sulaiman et al. (2003a)
	Peru7 (AY371282)		Sulaiman et al. (2003a)
	Peru8 (AY371283)		Sulaiman et al. (2003a)
	Peru11 (AY371286)	Peru12 (EF014428[a])[c]	Sulaiman et al. (2003a)
	Peru13 (EF014429)[a]		
	Peru15 (EF014431)[a]		
	CAF1 (DQ683746)		Breton et al. (2007)
	CAF2 (DQ683747)		Breton et al. (2007)
	CAF3 (DQ683748)		Breton et al. (2007)
	CAF4 (DQ683749 and DQ683757)		Breton et al. (2007)
	HAN1 (EF458627)		Espern et al. (2007)
	NIA1 (EF458628)		Espern et al. (2007)
	UG2145 (AF502396)[d]		Tumwine et al. (2002)
	Genotype 17 (EU140500)[a]		

[a]Unpublished.
[b]Genotype Q has 245 bp.
[c]Genotypes Peru11 and Peru12 differ in a nucleotide at position 3 in the large subunit rRNA gene (G vs. A).
[d]Genotype UG2145 was not named as a genotype but because it is a unique ITS sequence, we use here the isolate name as a genotype name.

Table **2.** *Enterocytozoon bieneusi* genotypes identified by the internal transcribed spacer (ITS) sequence and reported in humans and animals.

Hosts	Primary genotype name (GenBank accession number)	Synonyms (GenBank accession number)	References
Humans, beaver, fox, muskrat, and, raccoon	WL15 (AY237223)[a]	WL16 (AY237224) Peru14 (EF014430[b])	Sulaiman et al. (2003a, b)
Humans, beaver, cattle, dogs, falcons, fox, macaque, muskrat, pigs, raccoon	D (AF101200, DQ793213, DQ683751, DQ683755, and AF023245[c])	PigITS9 (AF348477) WL8 (AY237216) Peru9 (AY371284) PtEb VI (DQ885582) CEbC (EF139197)	Breton et al. (2007), Buckholt, Lee, and Tzipori (2002), Chalifoux et al. (2000), Lee (2007), Lobo et al. (2006a), Muller et al. (2008), Rinder et al. (1998), Sulaiman et al. (2003a, b)
Humans, birds, cattle, dogs	Peru6 (AY371281 and DQ154137)	PtEbI (DQ425107) PtEb VII (DQ885583)	Lobo et al. (2006a, b), Santín et al. (2005), Sulaiman et al. (2003a)
Humans, beaver, fox, muskrat, otter, pigs, and raccoon	EbpC (AF076042 and U61180[c])	E (AF135832)[d] WL13 (AY237221) WL17 (AY237225) Peru4 (AY371279)	Breitenmoser et al. (1999), Deplazes et al. (1996), Rinder et al. (2000), Sulaiman et al. (2003a, b)
Humans and cats	Peru10 (AY371285 and DQ836342)		Santín et al. (2006), Sulaiman et al. (2003a)
Humans and guinea pigs	Peru16 (EF014427)		Cama et al. (2007)
Humans, cats, dogs, and fox	WL11 (AY237219)	Peru5 (AY371280, DQ836344, and EU650271)	Santín et al. (2006, 2008), Sulaiman et al. (2003a, b)
Humans, cats, cattle, and dogs	Type IV (AF242478)	K (AF267141, DQ836343, DQ683752, DQ683756, and EU650272) Peru2 (AY371277) PtEb III (DQ885579) BEB5 (AY331009) BEB5-var (AY331010[b])[e]	Breton et al. (2007), Dengjel et al. (2001), Liguory et al. (1998), Lobo et al. (2006a), Santín et al. (2006, 2008), Sulaiman et al. (2003a, 2004)

[a]Genotypes WL15 and WL16/Peru14 differ in a nucleotide at position 25 in the large subunit rRNA gene (G vs. A).
[b]Unpublished.
[c]It was not named in the publication or GenBank.
[d]Genotypes E/WL13/Peru4 and WL17 differ in a nucleotide at position 25 in the large subunit rRNA gene (A vs. G).
[e]Genotypes K(AF267141 and DQ836343)/Peru2(AY371277)/PtEb III(DQ885579)/BEB5(AY331009) and BEB5-var(AY331010) differ in a nucleotide at position 1225 in the small subunit rRNA gene (A vs. T).

Table **3.** *Enterocytozoon bieneusi* genotypes identified by the internal transcribed spacer (ITS) sequence and reported in livestock (pigs and cattle).

Host	Primary genotype name (GenBank accession number)	Synonyms (GenBank accession number)	References
Cattle	I (AF135836)	BEB2 (AY331006) CEbE (EF139199)	Lee (2007), Rinder et al. (2000), Sulaiman et al. (2004)
	J (AF135837)	BEB1 (AY331005) PtEb X (DQ885586) CEbB (EF139196)	Lee (2007), Lobo et al. (2006a), Rinder et al. (2000), Sulaiman et al. (2004)
	M (AF267143)		Dengjel et al. (2001)
	N (AF267144)		Dengjel et al. (2001)
	PtEb XI (DQ885587)		Lobo et al. (2006a)
	BEB3 (AY331007)		Sulaiman et al. (2004)
	BEB4 (AY331008)		Sulaiman et al. (2004)
	BEB6 (EU153584)		Fayer, Santín, and Trout (2007)
	BEB7 (EU153585)		Fayer et al. (2007)
	CEbA (EF139195)		Lee (2007)
	CEbD (EF139198)		Lee (2007)
	CEbF (EF139194)		Lee (2007)
	4948 FL-2 2004 (DQ154136)		Santín et al. (2005)
Pigs	EbpB (AF076041)		Breitenmoser et al. (1999)
	EbpD (AF076043)		Breitenmoser et al. (1999)
	G (AF135834)		Rinder et al. (2000);
	H (AF135835)		Rinder et al. (2000);
	O (AF267145)		Dengjel et al. (2001)
	PigEBITS1 (AF348469)		Buckholt et al. (2002)
	PigEBITS2 (AF348470)		Buckholt et al. (2002)
	PigEBITS3 (AF348471)		Buckholt et al. (2002)
	PigEBITS4 (AF348472)		Buckholt et al. (2002)
	PigEBITS5 (AF348473)		Buckholt et al. (2002)
	PigEBITS6 (AF348474)		Buckholt et al. (2002)
	PigEBITS7 (AF348475)		Buckholt et al. (2002)
	PigEBITS8 (AF348476)		Buckholt et al. (2002)
Cattle and pigs	EbpA (AF076040)	F (AF135833)	Breitenmoser et al. (1999), Rinder et al. (2000)

Table **4.** *Enterocytozoon bieneusi* genotypes identified by the internal transcribed spacer (ITS) sequence and reported in companion animals (dogs and cats).

Host	Primary genotype name (GenBank accession number)	Synonyms (GenBank accession number)	References
Cats	L (AF267142)		Dengjel et al. (2001)
	PtEb IV (DQ885580)		Lobo et al. (2006a)
	PtEb VIII (DQ885584)		Lobo et al. (2006a)
	D-like (DQ836345)		Santín et al. (2006)
	AF118144[a]		Mathis, Breitenmoser, and Deplazes (1999)
Dogs	PtEb IX (DQ885585, AF059610, and EU650273)		Lobo et al. (2006a), Mathis et al. (1999), Santín et al. (2008)

[a]It was not named in the publication or GenBank.

Table **5.** *Enterocytozoon bieneusi* genotypes identified by the internal transcribed spacer (ITS) sequence and reported in miscellaneous hosts.

Host	Primary genotype Name (GenBank accession number)	Synonyms (GenBank accession number)	References
Beaver	WL7 (AY237215)		Sulaiman et al. (2003b)
	WL9 (AY237217)		Sulaiman et al. (2003b)
Birds	PtEb II (DQ425108)		Lobo et al. (2006b)
Kudu	PtEb V (DQ885581)		Lobo et al. (2006a)
Llama	P (AF267146)		Dengjel et al. (2001)
Marmoset	PtEb XII (DQ885588)		Lobo et al. (2006a)
Muskrat	WL4 (AY237212)	WL5 (AY237213)[a]	Sulaiman et al. (2003b)
	WL6 (AY237214)		Sulaiman et al. (2003b)
	WL10 (AY237218)		Sulaiman et al. (2003b)
	WL14 (AY237222)		Sulaiman et al. (2003b)
Raccoon	WL1 (AY237209)		Sulaiman et al. (2003b)
	WL2 (AY237210)		Sulaiman et al. (2003b)
	WL3 (AY237211)		Sulaiman et al. (2003b)
Beaver and otter	WL12 (AY237220)		Sulaiman et al. (2003b)

[a]Genotypes WL4 and WL5 differ in a nucleotide at position 29 in the large subunit rRNA gene (G vs. T).

names. For genotyping purposes based on ITS sequence data, only the 243 base pairs of the ITS region should be used. Base pairs associated with the small or large subunit rRNA should be excluded.

THE PROPOSED PLAN FOR FUTURE STUDIES

For the more distant future plan to improve source tracking and to be able to predict the host range and pathogenic potential of an isolate, studies are needed to identify new genetic markers and to determine whether a genotype identified by the ITS region corresponds with other genetic markers. New sets of markers could be very useful in the assessment of epidemiological relationships among *E. bieneusi* genotypes. The fact that one finds the same ITS sequence does not mean that the isolates are genetically identical. Tables 1–5 can also be found in http://www.biohealthbase.org/microsporidia where future information on *E. bieneusi* can be submitted.

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