Trends in Parasitology

CellPress
REVIEWS

Review

Host Specificity of *Enterocytozoon bieneusi* and Public Health Implications

Wei Li [1], Yaoyu Feng [2] and Monica Santin [3,*]

Enterocytozoon bieneusi **is the most common cause of human microsporidiosis and it also infects a wide range of mammals and birds worldwide. The role of animals in the transmission of this parasite to humans and its public health importance remain poorly elucidated. This review summarizes all *E. bieneusi* genotypes identified thus far based on sequence analysis of the ribosomal internal transcribed spacer (ITS) from specimens obtained from humans, domestic and wild animals, and water sources; it examines genotypes, host and geographical distribution, analyzes inter- and intragenotype group host specificity, and interprets the public health significance of genotype groups and major zoonotic genotypes, with the goal of improving our understanding of host specificity in *E. bieneusi* and its implications for interspecies and zoonotic transmission.**

E. bieneusi: Public Health Implications

The phylum Microsporidia contains obligate intracellular parasites that infect a wide range of vertebrate and invertebrate hosts [1,2]. Microsporidia currently comprises over 200 genera and 1500 species, with 17 of those species reported to cause infections in humans [3]. Of those 17 species, *E. bieneusi* is the most diagnosed species in humans; it is responsible for opportunistic infections in AIDS patients and other immunocompromised patients such as organ transplant recipients or cancer patients, as well as travelers, children, and the elderly [4]. *E. bieneusi* infections are mostly limited to the gastrointestinal tract, and diarrhea is the main clinical symptom; in those with immune suppression diarrhea can be persistent, resulting in wasting syndrome [4]. In addition to humans, *E. bieneusi* has been identified in numerous mammalian and avian hosts, raising concern on the importance of animal reservoirs in the epidemiology of this parasite [5]. It is interesting that, although *E. bieneusi* belongs to a family of microsporidia whose members exclusively infect aquatic hosts (fish and crustaceans), there is no record of *E. bieneusi* infecting any aquatic hosts [1,6]. So far, the epidemiology of *E. bieneusi*, including the sources and transmission routes, still remains elusive. Infections are thought to result from fecal–oral transmission of spores from infected humans or animals through contaminated food and water [3]. *E. bieneusi* produces environmentally resistant spores; spore morphology and the life cycle of the parasite are described in Box 1 and Figure 1. The detection of *E. bieneusi* spores in multiple water supplies, including irrigation waters used for crops, recreational waters, and effluents from wastewater treatment plants, supports the likelihood that water is a potential transmission vehicle for this parasite [7–9]. The presence of *E. bieneusi* in filter-feeding molluscs has been demonstrated, and although there is no current evidence of infection, it is possible that aquatic invertebrates could act as intermediate hosts or passive carriers important in the transmission of *E. bieneusi* [1,6]. Based upon the concern that water could be a source of microsporidian infection, the National Institute of Allergy and Infectious Diseases classified Microsporidia as priority pathogens in the Emerging Infectious Diseases/Pathogens category B[i]. Additionally, *E. bieneusi* was found in fresh retail produce, including raspberries, sprouts,

Highlights

High genetic diversity for *E. bieneusi* has been revealed using genetic polymorphisms at the ITS locus with almost 500 genotypes identified in humans, livestock, companion animals, wild mammals, birds, and water worldwide.

E. bieneusi genotypes vary in genetic or hereditary traits and 11 phylogenetic groups have been recognized using ITS genotyping data.

Genotypes in Group 1 and Group 2 have been found in a broad range of hosts including humans and are probably responsible for most zoonotic or cross-species *E. bieneusi* infections, whereas host adaptation seems to be more common in genotypes of Groups 3 to 11.

MLST and population genetic data have provided important insights into the host adaptation mechanism for some genotypes, but additional MLST tools and genomic data are required to elaborate the host specificity of *E. bieneusi* and its public health implications.

[1]Heilongjiang Key Laboratory for Zoonosis, College of Veterinary Medicine, Northeast Agricultural University, Harbin, China
[2]Key Laboratory of Zoonosis of Ministry of Agriculture, College of Veterinary Medicine, South China Agricultural University, Guangzhou, China
[3]Environmental Microbial and Food Safety Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, USA

*Correspondence: monica.santin-duran@ars.usda.gov (M. Santin).

Box 1. Morphology and Life Cycle of *E. bieneusi*

The infectious stage of *E. bieneusi* is the spore (Figure 1). It is small and ovoid-shaped (0.5 × 1.5 μm) and contains a single nucleus (monokaryon) [83]. Spores have a thick wall enabling long-term persistence and viability in the environment. The wall consists of an outer electron-dense exospore composed of glycoprotein, an electron-lucent endospore composed of protein and chitin or a chitin-like material, and an inner plasma membrane. The nucleus is surrounded by the cytoplasm which constitutes the infectious material (sporoplasm). The cytoplasm is enriched with prokaryote-like ribosomes and the infection-related organelles, including the membrane-bound polaroplast (atypical Golgi apparatus) that occupies one-third to one-half of the anterior portion of the spore, the polar tube (or polar filament) that emanates from the umbrella-shaped anchoring disk attaching at the apex of the spore and helically coils in the mid-to-posterior region of the spore, and the posterior vacuole. The polar tube, a unique organelle of the microsporidia, is considered the most important organelle involved in cell invasion. The life cycle of *E. bieneusi* starts with the ingestion of spores by a suitable host (Figure 1). After ingestion, the coiled polar tube discharges and injects the sporoplasm into host cells lining the digestive tract. Multiple intracellular stages develop (including meronts, multinucleate plasmodial cells, sporoblast cells, and mature spores). The mature spores excreted in feces of infected hosts can be ingested by new hosts and start a new round of intracellular replication.

and lettuce [10]. An *E. bieneusi* foodborne outbreak that caused gastrointestinal illness in more than 100 people was reported in Sweden in 2009 [11]; that no other pathogens but *E. bieneusi* genotype C were identified in the examined stools strongly implied that *E. bieneusi* was the causative agent [11]. Cucumber slices in cheese sandwiches and a salad were the most probable vehicles of transmission [11].

Diagnosis of Microsporidia, including *E. bieneusi*, was reviewed in detail by Ghosh *et al*. [12]. Microscopic detection of the spores is difficult due to their small size, and it is highly dependent on the expertise of the examiner for successful identification [5]. Currently, PCR-based methods are widely used to detect and molecularly characterize this parasite [5]. These methods offer not only higher specificity and sensitivity but also the advantage of further analysis for identification of genotypes. Nowadays, genotyping of *E. bieneusi* relies mainly on analysis of the polymorphisms of the ribosomal internal transcribed spacer (ITS) [13]. In addition to **ITS genotyping** (see Glossary), a new typing assay that includes several markers has become available, enabling improved understanding of the genetic diversity within this species [14]. *E. bieneusi* has a shrinking genome of ~6 megabases with different chromosome patterns among isolates, greatly reduced biological complexity, and a strong host dependence [15–17]. This review summarizes all *E. bieneusi* ITS genotyping data available, compares the levels of host specificity among ITS groups and major genotypes, and discusses the relationship between *E. bieneusi* ITS genotypes and phenotypic traits (host specificity and zoonotic potential) and the derived public health implications.

Molecular Typing of *E. bieneusi*

Most of the *E. bieneusi* genotyping studies have been based on the analysis of the polymorphism of the ITS nucleotide sequences. Currently, there are over 1600 full-length *E. bieneusi* ITS sequences deposited in GenBank obtained from over 650 studies. The ITS, an area of presumed nonfunctional RNA placed between ribosomal RNAs, is a fast-evolving genomic region that has shown a high degree of polymorphism, making it a good genotyping marker for elucidating intraspecific genetic diversity in *E. bieneusi* [5]. PCR primer pairs used for identification and genotyping of *E. bieneusi* isolates are summarized in Table S1 in the supplemental information online. Three of the nested primer pairs (MSP1/MSP2B and MSP3/MSP4B, EBITS3/EBITS4 and EBITS1/EBITS2.4, and AL4037/AL4039 and AL4038/ALA040) that amplify the entire ~243 bp ITS, as well as small portions of the conserved small- and large-subunit rRNA gene, have been used in over 95% of the published studies (Table S1). There has been some confusion naming *E. bieneusi* genotypes, therefore caution is encouraged when naming a new *E. bieneusi* genotype. The lack of standard nomenclature in the past

Glossary

Clonal population: a population of organisms with a common origin characterized by strong linkage disequilibrium among genetic loci with little or no genetic divergence among isolates in a host at any given time.

Epidemic population: a population characterized by relatively frequent recombination in which a clone emerges and becomes the predominant one for an extended period.

Host adaptation: the ability of a pathogen to circulate and cause disease in a host population. It is an indicator of the pathogen's fitness levels or the ability to adapt to its host environment, but might result in reduction in transmission of this pathogen to other hosts.

ITS genotyping: a typing approach based on hypermutation of the ribosomal ITS of an organism, and is used to discriminate among isolates from various sources.

Multilocus sequence typing (MLST): typically refers to the systematic sequencing of five to seven well-conserved, housekeeping loci with genetic polymorphisms within the genome of an organism. Different nucleotide sequences at a single locus represent distinct alleles, which define the allelic profile or sequence type of an isolate when the alleles of all loci are considered.

Population genetics: a subdivision of genetics, and a part of evolutionary biology, that studies genetic variation within and between populations and adaptation of organisms to host environments.

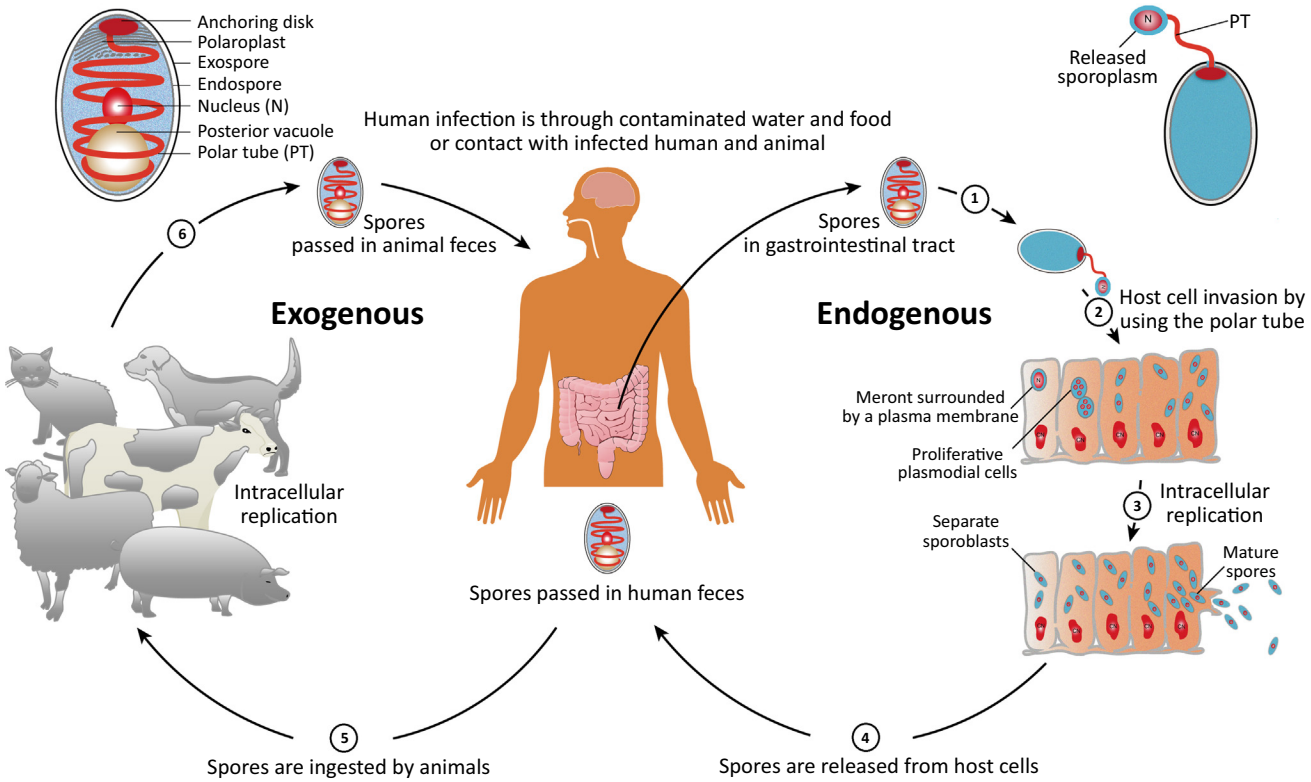

Figure 1. Diagrams of *Enterocytozoon bieneusi* Spore, Life Cycle, and Possible Sources of Human Infections. Humans can become infected with *E. bieneusi* via contact with infected humans and animals and contaminated water and food. After ingestion of the environmentally resistant spores by susceptible hosts the following events occur. (1) Spores germinate immediately under the extremely alkaline host gut conditions, and the polar tube discharges through eversion. (2) The sporoplasm is released into host cells through the cross-pierced polar tube and then develops into meronts coated by a simple plasma membrane. (3) The meronts multiply in direct contact with the host cytoplasm by multiple fission, with the formation of multinucleate plasmodial cells. The plasmodium undergoes sporogony to further divide and form sporoblast cells. (4) The separate sporoblasts go on to form mature spores with thick exospore layers that are dispersed freely within the host cell cytoplasm. Host cell damage associated with these intracellular stages is associated with disease aspects of *E. bieneusi*. Host cells then become distended, rupture, and release the mature spores. Some spores may infect other cells within the host while others are excreted in feces of the infected host. (5) Spores excreted in human feces can be ingested by animals. (6) Spores excreted in animal feces can be ingested by humans.

resulted in genotypes with identical ITS nucleotide sequences receiving multiple names [18]. This genotype name confusion is also attributable to the fact that polymorphisms in the flanking regions of the ITS, small and large subunits, were incorrectly considered when naming new genotypes [18]. A standardized nomenclature system for *E. bieneusi* ITS genotypes was proposed in a consensus paper to avoid confusion and to increase clarity regarding the terminology [18]. It indicated that only the polymorphisms at the ITS region should be used in designating new genotypes and that the first published genotype name should have priority when more than one name was given to identical ITS nucleotide sequences [18]. It also advised that genotype synonyms should be provided in subsequent publications and submissions of ITS sequences to GenBank [18]. All the nucleotide sequences currently available in GenBank that cover the entire ITS region were analyzed, and relevant information was summarized. According to the established naming convention [18] and an updated list of genotypes with synonyms (Table S2 in the supplemental information online), a total of 474 distinctive *E. bieneusi* genotypes have been validated (Table 1). However, it is important to remark that finding identical ITS sequences (genotype) would not denote that the isolates are fully or completely

Table 1. Distribution of *Enterocytozoon bieneusi* Genotypes in Phylogenetic Groups and Subgroups Based on the Phylogenetic Analysis of Nucleotide Sequences of the Ribosomal Internal Transcribed Spacer (ITS)

ITS groups[a]	Subgroups[a]	ITS genotypes
1 (*n* = 314)	1a (*n* = 78)	BEB15, CAF2, CAF3, CC2, CC3, CD1, CHALT1, CHN-RD3, CM12, CM13, CM15, CM16, CMB2, COS-V, COS-VI, COS-VII, CRep-3, D, D-like, EbfelA, ESH-02, ESH-03, ESH-04, ESH-05, ETMK1, ETMK2, ETMK3, ETMK4, Gorilla 1, Gorilla 2, Gorilla 4, Gorilla 5, Gorilla 6, Henan-II, Henan-V, HLJD-III, HNWW3, Horse6, Horse7, Horse8, Horse9, Horse10, Horse11, Ind5, JLD-I, JLD-III, KB-3, L, Macaque4, MH, NCF5, NCR2, Nig1, PigEBITS6, PigEBITS7, PigEBITS8, Peru8, Peru10, Peru11, Peru13, Peru15, Peru17, S6, SH1, SH6, T, V, WildBoar1, WL7, WL18, WL19, WL20, WL21, WR1, WR2, WR3, WR4, 4948FL-2
	1b/1c (*n* = 87)	A, B, BEB13, BEB16, CC4, CD2, CHN4, CHN11, CHN-B1, CHN-B2, CHN-B3, CHN-DC1, CHN-F1, CHN-R1, CHN-RD2, CHN-RD4, CHN-RR1, CHN-RR3, CM2, CM3, CMB1, Col01, Col02, COS-IV, CRep-1, CRep-2, CRep-4, EbCar1, EbCar2, EbCar3, HLJD-VI, HND-I, Ind2, Ind8, JLD-2, KB-1, KB-2, KB-4, KBAT3, Macaque3, MWC_d1, MWC_d2, NCD-1, NCD-2, NCR1, NIA1, Nig2, P, Peru3, Peru6, Peru7, PtEb II, PtEb IV, PtEb V, R, S, S1, S2, S3, S4, S5, S9, SC01, SC02, SC04, SC05, SC06, SC07, SC08, SCH2, SCH3, SH2, SH4, SH11, SW1, SW2, type III, type IV, type V, UG2145, WildBoar2, WildBoar3, WildBoar11, WL9, WL10, WL11, WL12
	1d (*n* = 44)	BEB19, CC-1, CHC5, CHG25, CHS10, CS-2, CS-3, CS-4, CS-5, E1, EbpC, GX1, GX2, GX3, HAN1, Henan-III, Henan-IV, HLJ-II, HLJD-II, HND-II, Horse5, JLD-IV, JLD-V, JLD-VI, NEC1, NEC2, NEC3, NEC4, NEC5, NECA1, NECA2, NECA3, NECA4, NECA5, NED1, NED2, NESH1, NESH2, NESH3, PigEb2, RWSH1, SH9, WL14, WL15
	1e (*n* = 87)	ABB2, BEB12, CD3, CD4, CEbD, CHC1, CHC3, CHC4, CHG6, CHG7, CHG8, CHG9, CHN7, CHN8, CHN12, CHN-RD1, CHN-RR2, CHP1, CHS11, CM8, CM10, CM11, CM14, CS-1, CS-6, CS-7, CS-8, CS-9, CS-10, CZ1, CZ2, CZ3, CZ4, CZ5, CZ6, EbpA, EbpB, EbpD, ESH-01, F1, G, Gorilla 7, H, HLJ-I, HLJ-IV, HLJ-CP1, HNWW5, Horse1, Horse4, IH, JLD-3, LW1, M, O, PigEb1, PigEb3, PigEb4, PigEb5, PigEb6, PigEb7, PigEb8, PigEb9, PigEb10, PigEb11, PigEb12, PigEb13, PigEb14, PigEb15, PigEb16, PigEb17, PigEBITS1, PigEBITS2, PigEBITS3, PigEBITS4, PigEBITS5, RWSH3, RWSH5, RWSH6, SH3, SH8, SH10, U, W, WildBoar5, WildBoar6, WildBoar8, XJH3
	1f (*n* = 5)	C, Gorilla 8, Peru16, Q, S8
	1g (*n* = 4)	CAF1, EbCar4, HLJ-III, SCH1
	1h (*n* = 7)	HNWW4, SHW2, WW1, WW2, WW3, WW4, WW5
	1i (*n* = 2)	CHN14, CHS17
2 (*n* = 94)	2a (*n* = 10)	BEB3, BEB8, BEB17, CHG10, CHG24, CM20, HLJD-IV, JLD-XII, KBAT2, KBAT4
	2b (*n* = 27)	BEB4, BEB9, BEB10, BEB11, BEB14, CEbA, CEbF, CHC2, CHC7, CHN2, CHN3, CHN5, CHN6, CHN9, CHN10, CHN13, DeerEb2, DeerEb4, DeerEb12, DeerEb13, I, I-like, J, JLD-VIII, KBAT1, N, PtEb XI
	2c (*n* = 57)	BEB6, BEB7, BEB18, BSH, CC1, CD6, CHC6, CHC8, CHG1, CHG2, CHG3, CHG5, CHG11, CHG13, CHG17, CHG18, CHG20, CHG22, CHS3, CHS6, CHS7, CHS8, CHS9, CHS13, CHS15, CHS16, CM5, CM7, CM9, CM21, COS-II, COS-III, HLJD-I, HLJD-V, HND-III, HND-IV, HNWW1, HNWW2, JLD-1, JLD-VII, JLD-IX, JLD-X, JLD-XI, JLD-XIII, JLD-XIV, NESH4, NESH5, NESH6, OEB1, OEB2, SX1, TEB1, TEB2, TEB3, TEB4, WR5, WR6
3 (*n* = 6)		PtEb VIII, WL4, WL6, WL22, WL23, WL25
4 (*n* = 6)		WL1, WL2, WL3, WL26, WW6, WW7
5 (*n* = 4)		CAF4, KB-6, KIN-3, PtEb XII
6 (*n* = 14)		CE01, Gorilla 3, Horse2, KB-5, macaque1, MAY1, Nig3, Nig4, Nig6, Nig7, XJH1, XJH4, YNH1, YNH2
7 (*n* = 4)		CD5, CM4, CM18, XH
8 (*n* = 9)		DeerEb1, DeerEb3, DeerEb5, DeerEb6, DeerEb7, DeerEb8, DeerEb9, DeerEb10, DeerEb11
9 (*n* = 4)		WR7, WR8, WR9, WR10
10 (*n* = 9)		CHB1, CHK1, CHK2, CSK1, EbRB, Horse3, S7, SCH4, WL24
11 (*n* = 7)		CD7, CD8, NED3, NED4, PtEb IX, WW8, WW9
Outlier (*n* = 3)		CSK2, Nig5, SW3

[a]Grouping and subgrouping of *E. bieneusi* isolates are based on phylogenetic analyses shown in Figure 2 and Figure S1.

genetically or phenotypically identical. Hence, it has been suggested that other genetic markers are needed to substantiate observations made by using ITS. Those markers will reveal whether genotype identification by ITS corresponds with typing determined by other markers and help to assess epidemiological relationships among *E. bieneusi* genotypes. A **multilocus sequence typing** (**MLST**; Box 2) tool for *E. bieneusi* has been developed; it targets three microsatellite markers and one minisatellite marker [14].

Phylogenetic Groups and Host Adaptation in *E. bieneusi*

To gain a better understanding of the genetic relationships among *E. bieneusi* isolates from various hosts or other sources, a comprehensive phylogenetic analysis of all valid ITS genotypes was conducted (Table 1; Figure 2, Key Figure). Eleven major genetic groups were recognized and named as Groups 1 to 11. Group 1 is the largest group containing 314 genotypes; it is further divided into nine subgroups designated as 1a to 1i (Table 1; see also Figure S1 in the supplemental information online). Within Group 2, the second largest group, three subgroups named 2a to 2c are identified (Table 1, Figure 2). Groups 3–11 contain fewer genotypes and were not further subdivided in subgroups. The grouping and subgrouping patterns were consistent with those previously reported in the literature [19–21].

Within Group 1, genotypes D, EbpC, and type IV are those most frequently found not only in humans but also in domestic and wild animals worldwide (Table 2). Indeed, those three genotypes have been found in most animals examined for *E. bieneusi* (Tables S3–S11 in the supplemental information online), suggesting a low level of host specificity and the potential for zoonotic or cross-species transmission. Additional Group 1 genotypes, such as Peru6, Peru8, and Peru11, have been regularly identified in both humans and animals (Tables S3–S11). In contrast, a few genotypes within Group 1 have restricted host ranges, suggesting that they are host specific. A genotype that appears to be human-specific is genotype B, found only in humans in Cameroon, The Netherlands, England, France, Nigeria, Tunisia, Australia, Germany, and Switzerland but not found in animals in any of those countries [22–30]. Other genotypes that appear to be restricted to specific hosts based on current data are genotypes gorilla 8, Peru7, Q, and S8 that seem to be specific to humans or non-human primates (NHPs) (Tables S3–S11), likewise, genotypes KB-1, KB-2, KB-3, and KB-4 to NHPs, genotypes EbpB, PigEBITS1, PigEBITS2, PigEBITS3, PigEBITS6, and PigEBITS8 to pigs, genotype CEbD to cattle, and genotype PtEb IV to cats. However, there are still limitations when talking about *E. bieneusi* host specificity because a very limited number of samples collected from the same

Box 2. MLST and Population Genetic Structure of *E. bieneusi*

MLST and **population genetics** analysis represent simple but robust tools for probing reproductive modes and transmission patterns [84]. An MLST tool including four markers (three microsatellites: MS1, MS3, and MS7, and one minisatellite: MS4) is available [14]. MLST was used to analyze human *E. bieneusi* isolates (almost all genotypes included have zoonotic potential) collected in various geographic locations to elucidate the potential occurrence of reproductive clonality and genetic segregation [43,85]. Clonality was widespread, and clear subpopulations with different genetic structures (epidemic versus clonal) and transmission routes (anthroponotic versus zoonotic) were found [43,85]. Additional studies that compared human *E. bieneusi* subpopulations with subpopulations identified in domestic pigs, foxes, and raccoon dogs, further support the existence of subdivisions and suggest **host adaptation** in some genotypes previously considered zoonotic [58,86]. **Clonal populations** contain mainly genotypes D, type IV, and EbpC and have a remarkably wide range of hosts and geographical regions which implies a high potential for cross-host species transmission. On the other hand, *E. bieneusi* genotypes with host-specific traits in **epidemic populations**, such as genotypes A, EbpA, and EbpB, have relatively limited host and geographic ranges (Table 2) [43,58,85]. However, multiple studies have failed to consistently amplify MS1, MS3, MS4, and MS7 in samples that were successfully genotyped using ITS [87–90], highlighting the need for additional reliable genetic markers that will successfully amplify isolates of all *E. bieneusi* genotypes to better understand host specificity and population genetic structure of the parasite.

Key Figure

Phylogenetic Relationship of All Valid *Enterocytozoon bieneusi* Internal Transcribed Spacer (ITS) Genotypes

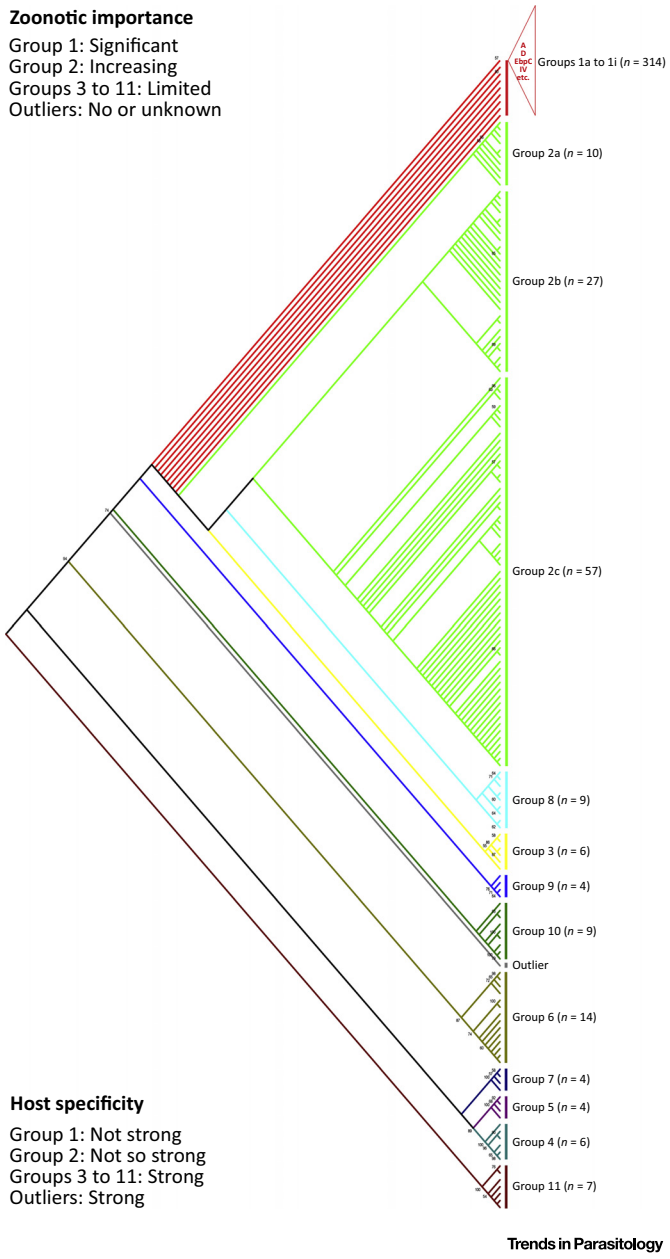

Figure 2. It was inferred by a neighbor-joining analysis implemented in Mega 7[ii] based on genetic distances calculated using the Kimura two-parameter model. Two exceptionally divergent genotypes, CSK2 (isolated from a kangaroo in China with GenBank accession no. KY706128) and SW3 (identified in stormwater in the USA with

(Figure legend continued on the bottom of the next page.)

Table 2. Host and Geographic Ranges of Some Common *Enterocytozoon bieneusi* Genotypes

Genotype	Host (location or source)[a]
A	Human (Cameroon, Gabon, Germany, Congo, India, Iran, Kenya, Netherlands, Niger, Nigeria, Peru, Portugal, Sao Tome, Switzerland, Thailand), NHP (Kenya), Dog (Spain), Bird (CZE)
BEB4	Human (China, CZE), NHP (China), Pig (China), Cattle (Argentina, Brazil, China, South Africa, USA), Yak (China)
BEB6	Human (China), NHP (China), Alpaca (China), Cattle (China, USA), Deer (China,), Goat (China, Peru), Sheep (Brazil, China, Sweden), Takin (China), Yak (China), Cat (China), Horse (China), Mouse (China), Bird (China), WW (China)
D	Human (Brazil, Cameroon, China, Congo, Gabon, India, Iran, Malawi, Netherlands, Niger, Nigeria, Peru, Poland, Portugal, Russia, Sao Tome, Spain, Thailand, Tunis, UK, Vietnam), NHP (China, Central African Republic, Kenya, Netherlands, Rwanda, Slovakia, USA), Pig (China, CZE, Japan, Thailand, USA), Wild boar (Austria, CZE, Slovakia), Cattle (Argentina, Brazil, China, Korea, South Africa, USA), Goat (China), Sheep (China), Takin (China), Cat (China, CZE, Poland, Slovakia, Thailand), Dog (China, Poland, Portugal), Donkey (China), Horse (Algeria, China, Colombia, CZE), Beaver (USA), Falcon (Abu Dhabi), Fox (China, Spain, USA), Hippo (China), Lion (China), Mouse (China, CZE, Germany, Poland), Muskrat (USA), Rabbit (China, Spain), Raccoon (China, USA), Raccoon dog (China), Otter (USA), Tiger (China), Bird (Brazil, China, Iran, Poland, United Arab Emirates), RW (China), WW (China, Spain, Tunisia)
EbpA	Human (China, CZE, Nigeria), NHP (Cameroon, China, Kenya, Rwanda, Slovakia), Pig (Brazil, China, CZE, Germany, Japan, Poland, Switzerland, Thailand, USA), Wild boar (Austria, China, CZE, Poland, Slovakia), Cattle (Brazil, China, Germany), Deer (China), Goat (China), Dog (China), Horse (CZE), Mouse (CZE), Bird (Brazil, China, CZE), WW (China)
EbpB	Pig (China, Switzerland), WW (China)
EbpC	Human (China, CZE, Iran, Peru, Thailand, Vietnam), NHP (China), Pig (China, Germany, Japan, Peru, Switzerland, Thailand), Wild boar (Austria, CZE, Poland, Slovakia), Cattle (Argentina, China), Deer (China), Goat (China), Sheep (China), Dog (China), Horse (China), Beaver (USA), Fox (China, USA), Mouse (China), Muskrat (USA), Otter (USA), Raccoon (USA), Panda (China), Bird (Iran), LW (China), RW (China), WW (China)
I	Human (China), NHP (China), Pig (Spain), Cattle (Argentina, Brazil, China, CZE, Germany, Korea, South Africa, USA), Deer (USA), Sheep (Brazil), Takin (China), Yak (China), Cat (China), Bat (Korea), Meerkat (China), Rabbit (China), WW (China)
J	Human (China), NHP (China), Alpaca (China), Cattle (Argentina, China, Germany, Korea, Portugal, USA), Deer (Australia, China, USA), Goat (China), Sheep (China), Yak (China), Donkey (China), Zebra (China), Bear (China), Meerkat (China), Bird (Germany, Iran), WW (China)
Type IV	Human (Cameroon, China, France, Gabon, Iran, Malawi, Netherlands, Niger, Nigeria, Peru, Portugal, Sao Tome, Uganda, UK), NHP (China), Cattle (Korea, Portugal, USA), Deer (Australia, China), Cat (China, Colombia, Germany, Japan, Portugal), Dog (China, Colombia), Bear (USA), Chipmunk (USA), Fox (China), Rabbit (China), Snake (China), Squirrel (USA), Vole (USA), Woodchuck (USA), Bird (Brazil, Spain), LW (China), WW (China, Ireland, Tunisia)

[a]CZE, Czech Republic; LW, lake water; NHP, non-human primates; RW, river water; WW, wastewater. The relevant data are collected based on those from Table S3 to Table S12 in this review.

locations have been examined for a broad range of animals and humans. Therefore, it is difficult to determine the actual extent of host specificity and how much of what we observe is actually related to data availability. Nonetheless, genotypes within Group 1 should be considered of public health importance based upon the widespread occurrence of genotypes such as D, EbpC, and type IV in a vast variety of hosts. There is a need for data covering multiple hosts at the same locations to clarify whether there is **host adaptation** in Group 1 genotypes.

Group 2 genotypes were once considered to be adapted to ruminants based on studies conducted solely in cattle [31]. However, the most frequently documented Group 2 genotypes in ruminants, BEB4, BEB6, I, and J (Tables S6 and S7 in the supplemental information online), have subsequently been found in other animals and humans (Table 2). BEB4 has been identified not only in cattle and yaks but also in humans, pigs, and NHPs; and J has been

GenBank accession no. KF591679), were considered as outliers and removed from analysis for better presentation of genetic clusters. The ITS tree is rooted with a highly divergent seq uence isolated from a dog (genotype PtEb IX, GenBank accession no. DQ885585). Only bootstrap values above 50% from 1000 pseudoreplicates are shown.

identified in alpacas, cattle, deer, goats, sheep, yaks, humans, bears, birds, donkeys, meerkats, zebras, and NHPs (Table 2). In addition, other genotypes in this group, such as BSH, CC1, CD6, CHN2, CHN5, CHN6, CHN9, CHN10, CM5, CM7, CM9, CM20, CM21, I-like, KBAT1, KBAT2, KBAT4, WR5, and WR6, have been isolated from multiple hosts as diverse as humans, NHPs, pigs, cats, dogs, pandas, mice, and bats (Tables S3, S4, S5, S8, and S10 in the supplemental information online). Therefore, support for host specificity no longer can be sustained for Group 2 genotypes. It also implies public health concern related to the zoonotic potential for some genotypes (notably BEB4, BEB6, I, and J) within this group.

Genotypes in Groups 3–11 appear to be more host-specific, but cautious interpretation is advised because little information is available for those groups. Among Group 3 genotypes, WL6, WL22, WL23, and WL25 are restricted to wild rodents in the USA, PtEb VIII to cats in Spain (Tables S3–S11 in the supplemental information online), and genotype WL4 has been reported only in wild rodents, wild deer, carnivores, and a cottontail in the USA (Tables S3–S11). Genotypes in Groups 4 (WL1, WL2, WL3, WL26, and WW6) and 9 (WR7, WR8, WR9, and WR10) appear to be adapted to wild carnivores and rodents (Tables S3–S11). The sole presence of genotypes DeerEb1, DeerEb3, and DeerEb5 to DeerEb11 in white-tailed deer in Group 8, and genotypes CD7, CD8, NED3, NED4, and WW8 in dogs in Group 11 seems to uphold host specificity for those genotypes (Tables S3–S11 in the supplemental information online). In Group 11, genotype PtEb IX has been identified mostly in dogs, with sporadic cases in cats, and a report in a wild badger (Table S8 and S10). Likewise, strong host specificity is suggested for Groups 5, 6, 7, and 10, as their genotypes have been found only in those hosts from which they were originally reported (Tables S3–S11). For example, genotypes CAF4 and KIN-3 in Group 5 and genotypes MAY1, Nig3, NIg4, Nig6, and Nig7 in Group 6, have been found only in humans. Likewise, genotypes KB-6 and PtEb XII in Group 5, gorilla 3 and KB-5 in Group 6, and CM18 and XH in Group 7 have been found only in NHPs; genotype CD5 in Group 7 in dogs; genotypes XJH1, XJH4, YNH1, and YNH2 in Group 6 and horse3 and SCH4 in Group 10 in horses; genotypes CHB1 and WL24 in Group 10 in carnivores; genotype CE01 in Group 6 in squirrels; and genotypes CHK1, CHK2, and CSK1 in Group 10 in kangaroos (Tables S3–S11). However, for the rest of the genotypes in Groups 6 (horse2 and macaque1), 7 (CM4), and 10 (S7), recent studies have added new host reports (Tables S3–S11). Two (Nig5 and CSK2) of the three outlier genotypes have been reported exclusively in an AIDS patient and a kangaroo, respectively (Tables S3–S11). There are some genotypes with unknown hosts because, thus far, they have been reported only in water sources, such as genotypes HNWW1 and HNWW2 in Group 2, WW7 in Group 4, EbRB in Group 10, WW9 in Group 11, and outlier genotype SW3 (Tables S3–S12). At this point, available data imply host adaptation in most genotypes in Groups 3 to 11, as well as the three outlier genotypes, suggesting limited zoonotic potential. Current findings strongly offer further evidence supporting that host adaptation would benefit from the use of additional genetic markers that will improve our understanding of population genetic traits in *E. bieneusi* (Box 2).

Enterocytozoon bieneusi Genotypes in Humans and Major Animal Groups

Humans

Genotypes belonging to Group 1 are mainly identified in humans, with genotypes A, D, EbpC, and type IV being the most prevalent (Table S3). However, most of the common genotypes detected in humans, such as D, EbpC, type IV, Peru6, Peru8, and Peru11, have been frequently documented in domestic and wild animals, and in different water sources (Tables S3–S12), suggesting the likelihood of zoonotic and waterborne transmission. Available data from MLST and population genetic analysis for genotypes D, EbpC, and type IV conducted in both humans and animals have upheld their zoonotic potential and emphasized the public health importance

of these genotypes (Box 2). It is worth mentioning the identification of the previously considered ruminant-adapted Group 2 genotypes (e.g., BEB4, BEB6, I, and J) in humans residing in the Czech Republic and China [32–34], implying a possibility of zoonotic transmission for those genotypes. The outlier genotype Nig5, Group 5 genotypes CAF4 and KIN-3, and Group 6 genotypes Nig3, Nig4, Nig6, and Nig7 may have robust host and geographic specificity because they have been identified in humans only in Africa (Tables S3–S11) [22,35–39]. Genotype MAY1 in Group 6 may also be very specific since it has been found only in a renal transplant recipient in France [40]. Some genotypes, such as S5, S7, and S9 reported in humans from The Netherlands, were thought to be human-specific, but their host and geographic ranges have been recently expanded, with S5 found in European badgers in Spain, S7 in a yak in China, and S9 in a red fox in Spain [23,41,42]. Unlike other prevalent genotypes D, EbpC, and type IV in Group 1, genotype A appears to have a relatively narrow host range, and the relevant population genetic data are indicative of some level of host specificity (Box 2). Host specificity may be present in Group 1 at a high level for some genotypes (e.g., IH and Nig2), judging by their unique presence in humans and genetic segregation of their multilocus genotypes (MLGs) from the zoonotic MLGs of genotypes D, EbpC, and type IV [43].

Non-human Primates

For NHPs, identification of both zoonotic and possibly host-adapted genotypes has been documented (Table S4). Most of the Group 1 genotypes (D, EbpA, EbpC, type IV, Henan-IV, O, Peru8, Peru11, etc.) found in NHPs are present also in humans (Tables S3 and S4). Group 2 genotypes BEB4, BEB6, I, and J have been found in NHPs [44–47] and also in humans [32–34], indicating a weak host specificity of some Group 2 genotypes with some cross-species capability [44–47]. Additional members of Group 2 found in NHPs include genotypes BEB8, BSH, CD6, CHG1, CM5, CM7, CM9, CM20, and CM21 (Table S4). *E. bieneusi* infections in NHPs also include genotypes KB-6 and PtEb XII in Group 5 [48,49] and genotypes gorilla 3, KB-5, and macaque1 in Group 6 [48,50,51], similar to what has been observed in humans with several potentially human-adapted genotypes in Groups 5 and 6 (CAF4, KIN-3, Nig3, Nig4, Nig6, and Nig7) (Table S3). Based on the present data (Tables S3–S11), genotypes gorilla 3, KB-5, KB-6, and PtEb XII are unique to NHPs, implying some degree of host specificity for Group 5 and Group 6. One exceptional case is that macaque1 was also reported in camels [52]. NHPs are the sole hosts of Group 7 genotypes CM18 and XH [45,46], while another Group 7 genotype CM4 can also infect sheep and goats [53] (Tables S3–S11).

In laboratory NHPs, only genotypes from Group 1 have been reported [54–57]. These animals are commonly bred indoors and have daily and frequent contact with their keepers, increasing the risk of zoonotic transmission. Besides genotypes in Group 1, a number of genotypes belonging to other genetic groups were found in wild and zoo NHPs [44,46–48]. Because wild and zoo NHPs are relatively isolated from human activities, it might have led to the circulation of host-adapted genotypes in those populations. However, the predominance of zoonotic genotype D in various types of NHPs in different studies indicates that they could be reservoirs of this genotype (Table S4).

Porcines

An overwhelming dominance of Group 1 genotypes has been observed in domestic pigs and wild boars, with genotypes D, EbpA, EbpC, G, H, and O being the most frequently identified (Table S5). Most of the genotypes found in pigs (e.g., CS-4, D, EbpA, EbpC, G, H, Henan-IV, PigEBITS5, PigEBITS7, BEB4, and I) have been identified in humans, suggesting an important role for pigs in transmission of *E. bieneusi* to humans (Tables S3 and S5). The zoonotic potential of some *E. bieneusi* isolates obtained from pigs has also been enhanced by population genetic

evidence [58]. Strikingly, one of the most popular zoonotic genotypes, type IV, has never been reported in pigs (Table S5). Only two reports have indicated the presence of Group 2 genotypes (BEB4, I, CHN9, and CHN10) in pigs [33,59]. The appearance of genotypes BEB4 and I in pigs seems to confirm a wider host range for genotypes in Group 2 than originally thought. However, some genotypes, CHN9 and CHN10, are thus far restricted to reports in pigs (Tables S3–S11). Overall, the frequent occurrence and high prevalence of zoonotic Group 1 genotypes in pigs are of significant concern from a public health point of view.

Ruminants

Genotypes identified in ruminants differed from those observed in humans, NHPs, and pigs that belong mostly to Group 1 and occasionally to other genetic groups. In ruminants, genotypes pertaining to Group 2, such as BEB4, BEB6, I, and J, are the ones more frequently reported (Tables S6 and S7). BEB4 seems to be more prevalent in cattle and yaks than in other ruminants (Table S6), while BEB6 is found primarily in sheep, goats, and deer (Table S7). However, genotypes I and J are widespread and reported frequently in various ruminant hosts (Tables S6 and S7). Although genotypes BEB4, BEB6, I, and J are still predominantly reported in ruminants, they have been shown to infect other hosts, including humans (Tables S3–S11). There has been a rapid rise in the number of new Group 2 genotypes originating from ruminants in recent years, but their host adaptation to ruminants remains to be elucidated (Tables S6 and S7).

Some of Group 1 genotypes have been identified in both domestic and wild ruminants (e.g., D, EbpC, type IV, Peru6, and CS-4) (Tables S6 and S7). In addition, several studies have reported Group 6 genotype macaque1 in camels in Algeria [52], Group 7 genotype CM4 in a sheep and a goat in China [53], and Group 3 genotype WL4 and Group 8 genotypes DeerEb1, DeerEb3, and DeerEb5 to DeerEb11 in wild deer in the USA [21,60]. In addition to ruminants, these unusual genotypes have never been reported in hosts other than CM4 and macaque1 in NHPs and WL4 in a variety of wildlife hosts (Tables S3–S11), suggesting the potential for a relatively high degree of host specificity. Identification in ruminants of genotypes identified also in humans is of public health importance and needs to be further explored even if the potential role of ruminants in the zoonotic transmission of *E. bieneusi* infections might not seem as important as that of NHPs and pigs.

Companion Animals

Reports of *E. bieneusi* are common in companion animals around the world (Table S8). Table S8 clearly shows that the frequently reported genotypes in humans, D and type IV, are those more frequently identified in cats. Group 2 genotypes, CC1, BEB6, and I, have also been found in cats [53,61]. In contrast, cats appear to have been the only host so far for Group 3 genotype PtEb VIII (Tables S3–S11). Genotype PtEb IX from the most likely host-adapted Group 11 has been occasionally found in cats (Table S8).

In dogs, a different genotype distribution pattern was observed, with PtEb IX being the dominant genotype reported in studies in Colombia, Japan, Switzerland, China, Portugal, and the USA [14,20,49,61–64]. This genotype has also been reported in cats in China and Poland [61,65] and a wild badger in Spain [42]. Some genotypes from Group 11 (CD7, CD8, NED3, NED4, and WW8) and Group 7 (CD5) have been reported only in dogs (Tables S3–S11). However, several reports indicated the presence of Group 1 genotypes with zoonotic potential such as D, EbpC, type IV, EbpA, O, and WL11 in dogs (Table S8). One (CD6) of the three Group 2 genotypes identified in dogs has been found in other hosts, including a NHP, dairy cattle, and goats, while the other two (CHN5 and CHN6) thus far have been found only in dogs (Tables S3–

S11). Due to the close and frequent contact of companion animals with humans, susceptible individuals should take precautions against the possible acquisition of *E. bieneusi* from cats and dogs because they could shed spores of potentially zoonotic genotypes. It seems that, because dogs most often carry the host-adapted genotype PtEb IX, the potential role of dogs in transmission of zoonotic genotypes should be less important than that of cats.

Equines

Limited information is available on *E. bieneusi* in equine animals. Among the genotypes identified, D and horse1 from Group 1 and horse2 from Group 6 are ones the most commonly reported (Table S9). Despite the clustering of horse1 into zoonotic Group 1, this genotype seems to have adapted well to equine animals since it has only been described in the studies conducted in horses in Colombia, China, Algeria, Czech Republic, and the USA [66–70], except for a single case in an NHP [71]. Likewise, horse2 is present mainly in horses and sporadically in a bear and two squirrels (Tables S3–S11). Genotypes in Group 6, YNH1, YNH2, XJH1, and XJH4, currently appear to have a restricted host range (Tables S3–S11). Nonrigid host specificity was observed in Group 2 genotypes identified in horses, BEB6, J, and CM7, as they have been reported also in a wide range of other hosts (Tables S3–S11). The Group 10 genotypes horse3 and SCH4 might be horse-specific as they only have horses as hosts (Tables S3–S11). The identification of Group 1 genotypes D and EbpC in horses indicates a potential public health risk to humans (Table S9).

Carnivores, Rodents, Lagomorphs, Marsupials, and Rare Hosts

Group 1 genotypes D, EbpC, and type IV possess a wide range of hosts (Table S10), suggesting that they are of public health importance. The hosts included in Table S10 can also harbor Group 2 genotypes BEB6, BEB8, I, I-like, J, KBAT1, KBAT2, KBAT4, WR5, and WR6, Group 3 genotypes WL4, WL6, WL22, WL23, and WL25, Group 4 genotypes WL1, WL2, WL3, WL26, and WW6, Group 6 genotypes CE01 and horse2, Group 9 genotypes WR7, WR8, WR9, and WR10, Group 10 genotypes CHB1, CHK1, CHK2, CSK1, and WL24, and Group 11 genotype PtEb IX (Table S10). An outlier genotype, CSK2, was identified in a kangaroo (Table S10). The host specificity of Group 2 is impaired by the distribution of its members BEB6 in chinchillas, I in rabbits, meerkats, and bats, and J in bears and meerkats (Table S10). The genotypes in Groups 3, 4, 6, 10, and 11 (e.g., WL1, WL2, WL3, WL4, or WL6) display strong host specificity, most of which seem to be restricted to carnivores, rodents, or lagomorphs (Tables S3–S11).

Birds

E. bieneusi genotypes identified in birds pertain mainly to Group 1 (A, CC-1, CHN-B1, CHN-B2, CHN-B3, CHP1, Col01, Col02, D, EbpA, EbpC, Henan-IV, M, Peru6, Peru8, Peru11, PtEb II, and type IV) and sporadically to Group 2 (BEB6 and J) (Table S11). Among those, A, BEB6, D, EbpA, EbpC, Henan-IV, J, M, Peru6, Peru8, Peru11, PtEb II, and type IV represent the common genotypes affecting humans (Tables S3 and S11). Table S11 explicitly exhibits the global and frequent appearance of Group 1 genotypes, D, EbpA, Peru6, and type IV, in free-ranging, captive, and domestic birds, implying a high zoonotic risk. The host specificity of Group 2 is again challenged by the identification of BEB6 and J in studies conducted in birds in Germany, China, and Iran [72–74].

E. bieneusi Genotypes in Water

Group 1 genotypes, D, EbpA, EbpC, Peru8, Peru11, and type IV, are the most frequent genotypes detected in urban wastewater, river water, and lake water (Table S12). In addition, some *E. bieneusi* genotypes from other genetic groups, BEB6, EbRB, HNWW1, HNWW2, I, J,

WL4, WW6, WW7, WW8, WW9, PtEb XI, and PtEb IX with different levels of host specificity, have been detected occasionally in water (Table S12). The predominance of wildlife-adapted Group 3 genotypes WL4 and WL6 has been observed in storm water in the USA [21]. High occurrence of potentially zoonotic genotypes D, EbpC, Peru8, Peru11, type IV, BEB6, I, and J, among others constitutes a risk for water security and public health.

Public Health Implications of Host Adaptation in *E. bieneusi*

Cross-Species Transmission and Zoonotic Origin

Genotyping, phylogeny, and host range analysis of human and animal *E. bieneusi* isolates allowed for identification of 11 phylogenetic groups with phenotypic differentiation in host-adaptive traits (Figure 2). Some of the Group 1 genotypes (D, EbpC, and type IV) with loose host specificity have great cross-species potential judging by their extremely broad host and geographic ranges as well as population genetic characteristics (Table 2, Box 2). Comparatively, some Group 1 genotypes (A, B, EbpA, EbpB, Peru7, Peru10, etc.) are probably adapted to a narrow range of hosts as inferred by the analysis of their host ranges or/and MLST data (Tables S3–S11; Box 2). Group 2 does not contain mainly ruminant-specific genotypes as previously thought. Current data indicate a broader host range for genotypes in this group than previously thought, increasing its importance for public health (Tables S3–S11). However, the genotypes in Groups 3 to 11 and the outliers seem to be adapted to specific hosts, which should be of very limited public health importance (Tables S3–S11).

Box 3. Infection Rates of *E. bieneusi* from Various Hosts and Sources

Humans

The infection rates of *E. bieneusi* in HIV-infected individuals are commonly in the range of 1.3% to 11.6%, aside from those higher rates reported in Nigeria (16.6%), Iran (28.4%), and Australia (85.2%) (Table S3). In immunocompetent individuals, low prevalences (<11.0%) are commonly noted (Table S3). The association of the infection rate of *E. bieneusi* with host immune status (HIV-positive or -negative) or clinical presentation (diarrheic or nondiarrheic) is normally not a tight one (Table S3).

NHPs

The significance of NHPs as potential sources of infection for humans has been increasingly appreciated. The infection rates of *E. bieneusi* varied from 1.8% to 46.2% in NHPs sampled from different sources (forest, farm, laboratory, park, zoo, etc.) and geographic regions (Table S4).

Porcines

The pig is one of the major domesticated animals worldwide. The infection rates of *E. bieneusi* in pigs vary considerably by regions from 10.0% to 93.7%, with no association with diarrhea (Table S5).

Ruminants

Ruminants are one of the most common hosts for *E. bieneusi* worldwide. The infection rates of *E. bieneusi* range from 2.2% to 37.6% in cattle, 1.1% to 22.2% in yaks, 4.4% to 69.3% in sheep, 21.8% to 28.8% in goats, and 4.1% to 35.9% in deer (Tables S6 and S7).

Companion Animals

The infection rates of *E. bieneusi* in companion animals fluctuate sharply worldwide, from 4.8% to 31.3% in cats and 1.7% to 20.5% in dogs (Table S8). A higher occurrence of *E. bieneusi* has been observed in stray cats than in pet cats, while the difference is not common in dogs (Table S8).

Other Hosts and Sources

The infection rates of *E. bieneusi* in equine animals vary from 1.6% to 30.9% (Table S9). There are distinctions in infection rates by other host types (carnivores, rodents, lagomorphs, marsupials, etc.) and regions (Table S10). The infection rates of *E. bieneusi* in birds fall in the range between 1.7% and 28.9% (Table S11). High detection rates of *E. bieneusi* in various water sources have been noted around the world (Table S12).

Among the animal hosts, NHPs and pigs often have relatively high infection rates (Box 3). They possibly represent the most important reservoirs for human *E. bieneusi* transmission because they have a high occurrence of zoonotic Group 1 genotypes (Tables S3–S5). In addition, other mammals (ruminants, cats, dogs, horses, carnivores, rodents, etc.) and birds could also be of public health importance because they are usually infected with some zoonotic genotypes (Tables S6–S11). Although there is still no direct evidence linking human infections to *E. bieneusi* of animal origin, frequent contact of humans with household animals (pigs and guinea pigs) has been considered a significant risk factor for zoonotic transmission [75,76]. Humans could also acquire *E. bieneusi* infections through the anthroponotic route as previously described [77,78]. The potential zoonotic or cross-species transmission of some *E. bieneusi* genotypes, notably D, EbpC, and type IV, between wildlife, domestic animals, and humans, as illustrated in Figure 3, is important to be aware of in public safety and veterinary health.

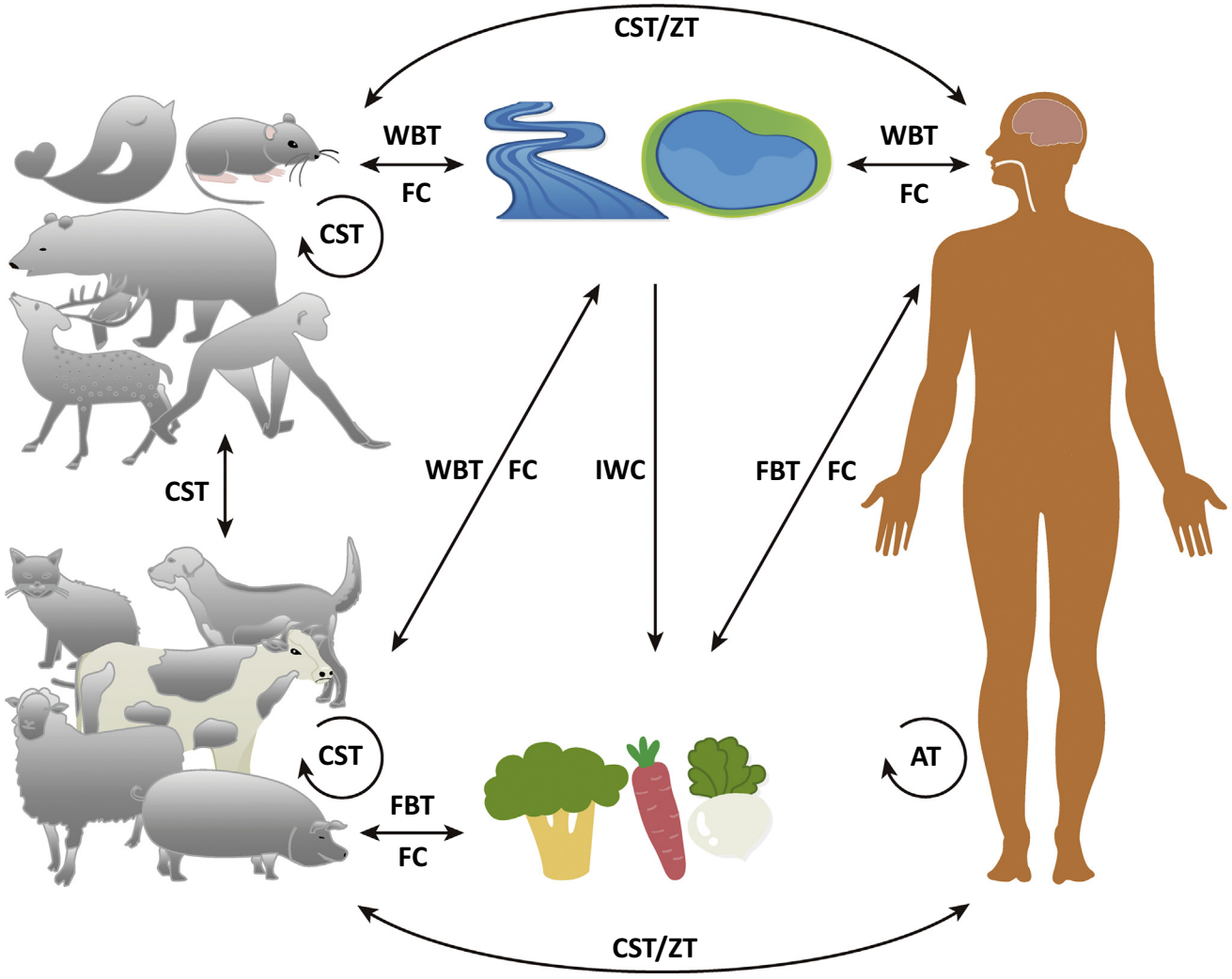

Figure 3. Transmission Routes for *Enterocytozoon bieneusi*. Diagram on potential anthroponotic (AT), zoonotic (ZT), cross-species (CST), waterborne (WBT), or foodborne (FBT) transmission of *E. bieneusi*. Surface water and food could be contaminated with the fecal materials (FC) containing *E. bieneusi* spores from humans and domestic and wild animals. Crops could also be contaminated with irrigation water (IWC) containing *E. bieneusi* spores.

Waterborne and Foodborne Transmission

Zoonotic and potentially host-adapted *E. bieneusi* genotypes have been detected in different water sources (Table S12). The repeated identification of human-pathogenic *E. bieneusi* genotypes, including D, EbpC, type IV, Peru8, and Peru11, among others, in surface water demonstrates that waterborne zoonotic transmission of microsporidiosis is a strong possibility [8,9,79–81]. Animals could also become infected with *E. bieneusi* via waterborne transmission (Figure 3) as previously described [51]. In addition, the detection of *E. bieneusi* spores in berries, sprouts, and vegetables at retail markets, and the presence of human-pathogenic *E. bieneusi* genotypes D, I, J, and type IV in milk, constitute potential risks for transmission via foods (Figure 3) [10,82]. Indeed, a foodborne parasitic outbreak has been confirmed to be related to *E. bieneusi* infection [11].

Concluding Remarks

A wide genetic variation has been observed within *E. bieneusi* isolates obtained from humans, domestic and wild animals, and surface water worldwide. Analysis of the ITS-based epidemiologic and genetic data to determine genetic diversity, zoonotic or cross-species potential, and host specificity of *E. bieneusi* indicates that, although *E. bieneusi* has the capability to infect a broad range of hosts there is a certain level of host specificity occurring mainly for genotypes in Groups 3 to 11. Host specificity for genotypes in Group 1 and 2 appears more questionable as a wide host range was reported for many of those genotypes. While progress has been significant, there are still important questions that need to be addressed (see Outstanding Questions). The potential factors that modulate adaptation of different *E. bieneusi* genotypes to specific host/s remain uncertain. Current MLST and population genetic data have provided new insights into the host adaptation mechanisms and resolved some issues on host range variation. However, the existing MLST tool is not applicable to all *E. bieneusi* isolates, maybe because hypermutation in the genome prevents some genotypes from amplifying. Therefore, application of whole-genome sequencing to more isolates is necessary to identify more effective polymorphic markers to better understand *E. bieneusi*'s host adaptation and epidemiology. Additional MLST tools and genomic data will assist us to reveal *E. bieneusi* population structure and its relation to host, socioeconomic, geographical, and other epidemiological risk factors to fully exploit the public health implications of host specificity.

Outstanding Questions

How do the limited, or lack of, epidemiologic and genetic data from humans and animals in the same geographic regions impact our ability to comprehend *E. bieneusi*'s host specificity?

Can novel MLST tools be effectively developed to type all *E. bieneusi* isolates?

How can we successfully obtain more comparative genomic data to explore new genetic markers, even when no *in vitro* culture method is available for *E. bieneusi*?

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31425025). W.L. was supported by grant 2017FYD0501302 from the National Key Research and Development Program of China; the founder has no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

Resources

[i]www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens

[ii]www.megasoftware.net/

Supplemental Information

Supplemental information associated with this article can be found online at https://doi.org/10.1016/j.pt.2019.04.004.

References

1. Stentiford, G.D. *et al.* (2016) Microsporidia – emergent pathogens in the global food chain. *Trends Parasitol.* 32, 336–348
2. Vavra, J. and Lukes, J. (2013) Microsporidia and 'the art of living together'. *Adv. Parasitol.* 82, 253–319
3. Weiss, L.M. and Becnel, J.J. (2014) *Microsporidia: Pathogens of Opportunity*. (1st edn), Wiley Blackwell
4. Matos, O. *et al.* (2012) Epidemiology of *Enterocytozoon bieneusi* infection in humans. *J. Parasitol. Res.* 2012, 981424
5. Santin, M. and Fayer, R. (2011) Microsporidiosis: *Enterocytozoon bieneusi* in domesticated and wild animals. *Res. Vet. Sci.* 90, 363–371
6. Stentiford, G.D. *et al.* (2013) Microsporidia: diverse, dynamic, and emergent pathogens in aquatic systems. *Trends Parasitol.* 29, 567–578

7. Ben Ayed, L. *et al.* (2012) Survey and genetic characterization of wastewater in Tunisia for *Cryptosporidium* spp., *Giardia duodenalis*, *Enterocytozoon bieneusi*, *Cyclospora cayetanensis* and *Eimeria* spp. *J. Water Health*, 10, 431–444
8. Li, N. *et al.* (2012) Molecular surveillance of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* by genotyping and subtyping parasites in wastewater. *PLoS Negl. Trop. Dis.* 6, e1809
9. Galvan, A.L. *et al.* (2013) Molecular characterization of human-pathogenic microsporidia and *Cyclospora cayetanensis* isolated from various water sources in Spain: a year-long longitudinal study. *Appl. Environ. Microbiol.* 79, 449–459
10. Jedrzejewski, S. *et al.* (2007) Quantitative assessment of contamination of fresh food produce of various retail types by human-virulent microsporidian spores. *Appl. Environ. Microbiol.* 73, 4071–4073
11. Decraene, V. *et al.* (2012) First reported foodborne outbreak associated with microsporidia, Sweden, October 2009. *Epidemiol. Infect.* 140, 519–527
12. Ghosh, K. *et al.* (2014) Laboratory diagnosis of Microsporidia. In *Microsporidia: Pathogens of Opportunity* (Weiss, L.M. and Becnel, J.J., eds), pp. 421–456, Wiley Blackwell
13. Santin, M. *et al.* (2015) Enterocytozoon bieneusi. In *Biology of Foodborne Parasites* (Xiao, L., ed.), pp. 149–174, CRC Press (Taylor & Francis Group)
14. Feng, Y. *et al.* (2011) Development of a multilocus sequence typing tool for high-resolution genotyping of *Enterocytozoon bieneusi*. *Appl. Environ. Microbiol.* 77, 4822–4828
15. Akiyoshi, D.E. *et al.* (2009) Genomic survey of the non-cultivatable opportunistic human pathogen, *Enterocytozoon bieneusi*. *PLoS Pathog.* 5, e1000261
16. Keeling, P.J. *et al.* (2010) The reduced genome of the parasitic microsporidian *Enterocytozoon bieneusi* lacks genes for core carbon metabolism. *Genome Biol. Evol.* 2, 304–309
17. Corradi, N. *et al.* (2007) Patterns of genome evolution among the microsporidian parasites *Encephalitozoon cuniculi*, *Antonospora locustae* and *Enterocytozoon bieneusi*. *PLoS One*, 2, e1277
18. Santin, M. and Fayer, R. (2009) *Enterocytozoon bieneusi* genotype nomenclature based on the internal transcribed spacer sequence: a consensus. *J. Eukaryot. Microbiol.* 56, 34–38
19. Fiuza, V.R.D.S. *et al.* (2016) New findings of *Enterocytozoon bieneusi* in beef and dairy cattle in Brazil. *Vet. Parasitol.* 216, 46–51
20. Li, W. *et al.* (2015) Prevalence and genetic characteristics of *Cryptosporidium*, *Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, China. *Vet. Parasitol.* 208, 125–134
21. Guo, Y. *et al.* (2014) Host specificity and source of *Enterocytozoon bieneusi* genotypes in a drinking source watershed. *Appl. Environ. Microbiol.* 80, 218–225
22. Breton, J. *et al.* (2007) New highly divergent rRNA sequence among biodiverse genotypes of *Enterocytozoon bieneusi* strains isolated from humans in Gabon and Cameroon. *J. Clin. Microbiol.* 45, 2580–2589
23. ten Hove, R.J. *et al.* (2009) Characterization of genotypes of *Enterocytozoon bieneusi* in immunosuppressed and immunocompetent patient groups. *J. Eukaryot. Microbiol.* 56, 388–393
24. Sadler, F. *et al.* (2002) Genotyping of *Enterocytozoon bieneusi* in AIDS patients from the north west of England. *J. Infect.* 44, 39–42
25. Liguory, O. *et al.* (1998) Determination of types of *Enterocytozoon bieneusi* strains isolated from patients with intestinal microsporidiosis. *J. Clin. Microbiol.* 36, 1882–1885
26. Liguory, O. *et al.* (2001) Evidence of different *Enterocytozoon bieneusi* genotypes in patients with and without human immunodeficiency virus infection. *J. Clin. Microbiol.* 39, 2672–2674
27. Ojuromi, O.T. *et al.* (2012) Identification and characterization of microsporidia from fecal samples of HIV-positive patients from Lagos, Nigeria. *PLoS One*, 7, e35239
28. Chabchoub, N. *et al.* (2012) Genotype identification of *Enterocytozoon bieneusi* isolates from stool samples of HIV-infected Tunisian patients. *Parasite*, 19, 147–151
29. Stark, D. *et al.* (2009) Limited genetic diversity among genotypes of *Enterocytozoon bieneusi* strains isolated from HIV-infected patients from Sydney, Australia. *J. Med. Microbiol.* 58, 355–357
30. Rinder, H. *et al.* (1997) Evidence for the existence of genetically distinct strains of *Enterocytozoon bieneusi*. *Parasitol. Res.* 83, 670–672
31. Thellier, M. and Breton, J. (2008) *Enterocytozoon bieneusi* in human and animals, focus on laboratory identification and molecular epidemiology. *Parasite*, 15, 349–358
32. Sak, B. *et al.* (2011) Unapparent microsporidial infection among immunocompetent humans in the Czech Republic. *J. Clin. Microbiol.* 49, 1064–1070
33. Zhang, X. *et al.* (2011) Identification and genotyping of *Enterocytozoon bieneusi* in China. *J. Clin. Microbiol.* 49, 2006–2008
34. Wang, L. *et al.* (2013) Concurrent infections of *Giardia duodenalis*, *Enterocytozoon bieneusi*, and *Clostridium difficile* in children during a cryptosporidiosis outbreak in a pediatric hospital in China. *PLoS Negl. Trop. Dis.* 7, e2437
35. Wumba, R. *et al.* (2012) Epidemiology, clinical, immune, and molecular profiles of microsporidiosis and cryptosporidiosis among HIV/AIDS patients. *Int. J. Gen. Med.* 5, 603–611
36. Akinbo, F.O. *et al.* (2012) Molecular epidemiologic characterization of *Enterocytozoon bieneusi* in HIV-infected persons in Benin City, Nigeria. *Am. J. Trop. Med. Hyg.* 86, 441–445
37. Ayinmode, A.B. *et al.* (2014) *Cryptosporidium hominis* subtypes and *Enterocytozoon bieneusi* genotypes in HIV-infected persons in Ibadan, Nigeria. *Zoonoses Public Health*, 61, 297–303
38. Akinbo, F.O. *et al.* (2013) Unusual *Enterocytozoon bieneusi* genotypes and *Cryptosporidium hominis* subtypes in HIV-infected patients on highly active antiretroviral therapy. *Am. J. Trop. Med. Hyg.* 89, 157–161
39. Lobo, M.L. *et al.* (2014) *Cryptosporidium* spp., *Giardia duodenalis*, *Enterocytozoon bieneusi* and other intestinal parasites in young children in Lobata province, Democratic Republic of Sao Tome and Principe. *PLoS One*, 9, e97708
40. Pomares, C. *et al.* (2012) A new and highly divergent *Enterocytozoon bieneusi* genotype isolated from a renal transplant recipient. *J. Clin. Microbiol.* 50, 2176–2178
41. Li, J. *et al.* (2015) Molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* in captive wildlife at Zhengzhou zoo, China. *J. Eukaryot. Microbiol.* 62, 833–839
42. Santin, M. *et al.* (2018) Molecular characterization of *Enterocytozoon bieneusi* in wild carnivores in Spain. *J. Eukaryot. Microbiol.* 65, 468–474
43. Li, W. *et al.* (2013) Multilocus sequence typing of *Enterocytozoon bieneusi*: lack of geographic segregation and existence of genetically isolated sub-populations. *Infect. Genet. Evol.* 14, 111–119
44. Karim, M.R. *et al.* (2014) Genetic polymorphism and zoonotic potential of *Enterocytozoon bieneusi* from nonhuman primates in China. *Appl. Environ. Microbiol.* 80, 1893–1898
45. Du, S.Z. *et al.* (2015) *Cryptosporidium* spp., *Giardia intestinalis*, and *Enterocytozoon bieneusi* in captive non-human primates in Qinling mountains. *Korean J. Parasitol.* 53, 395–402
46. Karim, M.R. *et al.* (2015) Predomination and new genotypes of *Enterocytozoon bieneusi* in captive nonhuman primates in zoos in China: high genetic diversity and zoonotic significance. *PLoS One*, 10, e0117991
47. Yu, F. *et al.* (2017) High prevalence of *Enterocytozoon bieneusi* zoonotic genotype D in captive golden snub-nosed monkey (*Rhinopithecus roxellanae*) in zoos in China. *BMC Vet. Res.* 13, 158
48. Li, W. *et al.* (2011) *Cyclospora papionis*, *Cryptosporidium hominis*, and human-pathogenic *Enterocytozoon bieneusi* in captive baboons in Kenya. *J. Clin. Microbiol.* 49, 4326–4329

49. Lobo, M.L. *et al.* (2006) Genotypes of *Enterocytozoon bieneusi* in mammals in Portugal. *J. Eukaryot. Microbiol.* 53, S61–S64
50. Sak, B. *et al.* (2013) Long-term monitoring of microsporidia, *Cryptosporidium* and *Giardia* infections in western Lowland Gorillas (*Gorilla gorilla gorilla*) at different stages of habituation in Dzanga Sangha Protected Areas, Central African Republic. *PLoS One*, 8, e71840
51. Ye, J. *et al.* (2012) Anthroponotic enteric parasites in monkeys in public park, China. *Emerg. Infect. Dis.* 18, 1640–1643
52. Baroudi, D. *et al.* (2018) Divergent *Cryptosporidium parvum* subtype and *Enterocytozoon bieneusi* genotypes in dromedary camels in Algeria. *Parasitol. Res.* 117, 905–910
53. Shi, K. *et al.* (2016) Molecular survey of *Enterocytozoon bieneusi* in sheep and goats in China. *Parasites Vectors*, 9, 23
54. Chalifoux, L.V. *et al.* (2000) *Enterocytozoon bieneusi* as a cause of proliferative serositis in simian immunodeficiency virus-infected immunodeficient macaques (*Macaca mulatta*). *Arch. Pathol. Lab. Med.* 124, 1480–1484
55. Drosten, C. *et al.* (2005) Interspecies transmission of *Enterozytozoon bieneusi* supported by observations in laboratory animals and phylogeny. *Med. Microbiol. Immunol.* 194, 207–209
56. Ye, J. *et al.* (2014) Occurrence of human-pathogenic *Enterocytozoon bieneusi*, *Giardia duodenalis* and *Cryptosporidium* genotypes in laboratory macaques in Guangxi, China. *Parasitol. Int.* 63, 132–137
57. Yang, H. *et al.* (2017) Molecular characterization of *Enterocytozoon bieneusi* isolates in laboratory macaques in north China: zoonotic concerns. *Parasitol. Res.* 116, 2877–2882
58. Wan, Q. *et al.* (2016) Clonal evolution of *Enterocytozoon bieneusi* populations in swine and genetic differentiation in subpopulations between isolates from swine and humans. *PLoS Negl. Trop. Dis.* 10, e0004966
59. Galvan-Diaz, A.L. *et al.* (2014) Microsporidia detection and genotyping study of human pathogenic *E. bieneusi* in animals from Spain. *PLoS One*, 9, e92289
60. Santin, M. and Fayer, R. (2015) *Enterocytozoon bieneusi*, *Giardia*, and *Cryptosporidium* infecting white-tailed deer. *J. Eukaryot. Microbiol.* 62, 34–43
61. Karim, M.R. *et al.* (2014) Genetic diversity in *Enterocytozoon bieneusi* isolates from dogs and cats in China: host specificity and public health implications. *J. Clin. Microbiol.* 52, 3297–3302
62. Santin, M. *et al.* (2008) *Enterocytozoon bieneusi* genotypes in dogs in Bogota, Colombia. *Am. J. Trop. Med. Hyg.* 79, 215–217
63. Abe, N. *et al.* (2009) Molecular evidence of *Enterocytozoon bieneusi* in Japan. *J. Vet. Med. Sci.* 71, 217–219
64. Mathis, A. *et al.* (1999) Detection of new *Enterocytozoon* genotypes in faecal samples of farm dogs and a cat. *Parasite*, 6, 189–193
65. Piekarska, J. *et al.* (2017) Zoonotic microsporidia in dogs and cats in Poland. *Vet. Parasitol.* 246, 108–111
66. Santin, M. *et al.* (2010) A zoonotic genotype of *Enterocytozoon bieneusi* in horses. *J. Parasitol.* 96, 157–161
67. Qi, M. *et al.* (2016) *Enterocytozoon bieneusi* genotypes in grazing horses in China and their zoonotic transmission potential. *J. Eukaryot. Microbiol.* 63, 591–597
68. Laatamna, A.E. *et al.* (2015) Microsporidia and *Cryptosporidium* in horses and donkeys in Algeria: detection of a novel *Cryptosporidium hominis* subtype family (Ik) in a horse. *Vet. Parasitol.* 208, 135–142
69. Wagnerova, P. *et al.* (2012) *Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* in horses kept under different management systems in the Czech Republic. *Vet. Parasitol.* 190, 573–577
70. Wagnerova, P. *et al.* (2016) *Cryptosporidium parvum* and *Enterocytozoon bieneusi* in American mustangs and chincoteague ponies. *Exp. Parasitol.* 162, 24–27
71. Zhong, Z. *et al.* (2017) Multilocus genotyping of *Enterocytozoon bieneusi* derived from nonhuman primates in southwest China. *PLoS One*, 12, e0176926
72. Reetz, J. *et al.* (2002) First detection of the microsporidium *Enterocytozoon bieneusi* in non-mammalian hosts (chickens). *Int. J. Parasitol.* 32, 785–787
73. Zhao, W. *et al.* (2016) Genotyping of *Enterocytozoon bieneusi* (Microsporidia) isolated from various birds in China. *Infect. Genet. Evol.* 40, 151–154
74. Pirestani, M. *et al.* (2013) Molecular characterization and genotyping of human related microsporidia in free-ranging and captive pigeons of Tehran, Iran. *Infect. Genet. Evol.* 20, 495–499
75. Cama, V.A. *et al.* (2007) Transmission of *Enterocytozoon bieneusi* between a child and guinea pigs. *J. Clin. Microbiol.* 45, 2708–2710
76. Wang, L. *et al.* (2013) Zoonotic *Cryptosporidium* species and *Enterocytozoon bieneusi* genotypes in HIV-positive patients on antiretroviral therapy. *J. Clin. Microbiol.* 51, 557–563
77. Leelayoova, S. *et al.* (2005) Transmission of *Enterocytozoon bieneusi* genotype a in a Thai orphanage. *Am. J. Trop. Med. Hyg.* 73, 104–107
78. Pagornrat, W. *et al.* (2009) Carriage rate of *Enterocytozoon bieneusi* in an orphanage in Bangkok, Thailand. *J. Clin. Microbiol.* 47, 3739–3741
79. Huang, C. *et al.* (2017) Environmental transport of emerging human-pathogenic *Cryptosporidium* species and subtypes through combined sewer overflow and wastewater. *Appl. Environ. Microbiol.* 83, e00682-17
80. Izquierdo, F. *et al.* (2011) Detection of microsporidia in drinking water, wastewater and recreational rivers. *Water Res.* 45, 4837–4843
81. Hu, Y. *et al.* (2014) Occurrence, source, and human infection potential of *Cryptosporidium* and *Enterocytozoon bieneusi* in drinking source water in Shanghai, China, during a pig carcass disposal incident. *Environ. Sci. Technol.* 48, 14219–14227
82. Lee, J.H. (2008) Molecular detection of *Enterocytozoon bieneusi* and identification of a potentially human-pathogenic genotype in milk. *Appl. Environ. Microbiol.* 74, 1664–1666
83. Wasson, K. and Peper, R.L. (2000) Mammalian microsporidiosis. *Vet. Pathol.* 37, 113–128
84. Tibayrenc, M. and Ayala, F.J. (2012) Reproductive clonality of pathogens: a perspective on pathogenic viruses, bacteria, fungi, and parasitic protozoa. *Proc. Natl. Acad. Sci. U. S. A.* 109, E3305–E3313
85. Li, W. *et al.* (2012) Population genetic analysis of *Enterocytozoon bieneusi* in humans. *Int. J. Parasitol.* 42, 287–293
86. Li, W. *et al.* (2016) Genetic variation of mini- and microsatellites and a clonal structure in *Enterocytozoon bieneusi* population in foxes and raccoon dogs and population differentiation of the parasite between fur animals and humans. *Parasitol. Res.* 115, 2899–2904
87. Zou, Y. *et al.* (2018) Prevalence and genotypes of *Enterocytozoon bieneusi* in pigs in southern China. *Infect. Genet. Evol.* 66, 52–56
88. Chen, D. *et al.* (2018) Prevalence and multi-locus genotypes of *Enterocytozoon bieneusi* in black-boned sheep and goats in Yunnan Province, southwestern China. *Infect. Genet. Evol.* 65, 385–391
89. Wu, Y. *et al.* (2018) Occurrence and molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* from Tibetan sheep in Gansu, China. *Infect. Genet. Evol.* 64, 46–51
90. Tang, C. *et al.* (2018) Genetic diversity within dominant *Enterocytozoon bieneusi* genotypes in pre-weaned calves. *Parasites Vectors*, 11, 170