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Population genetic analysis suggests genetic recombination is responsible for increased zoonotic potential of *Enterocytozoon bieneusi* from ruminants in China

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ABSTRACT

Enterocytozoon bieneusi is a zoonotic pathogen with worldwide distribution. Among the 11 established groups of *E. bieneusi* genotypes based on phylogenetic analysis of the ribosomal internal transcribed spacer (*ITS*), the human-infective potential and population genetics of the Group 1 genotypes from diverse hosts are well characterized. In contrast, Group 2 genotypes from ruminants have unclear population genetics, leading to poor understanding of their host range and zoonotic potential. In this study, we sequence-characterized 121 Group 2 isolates from dairy cattle, beef cattle, yaks, Tibetan sheep, golden takins, and deer from China at five genetic loci (*ITS*, MS1, MS3, MS4 and MS7), comparing with data from 113 Group 1 isolates from nonhuman primates. Except for MS7, most of the genetic loci produced efficient PCR amplification and high nucleotide identity between Groups 1 and 2 of *E. bieneusi* genotypes. In population genetic analyses of the sequence data, a strong linkage disequilibrium was observed among these genetic loci in the overall Group 2 population. The individual *ITS* genotypes (I, J and BEB4) within Group 2, however, had reduced linkage disequilibrium and increased genetic exchanges among isolates. There was only partial genetic differentiation between Group 1 and Group 2 genotypes, with some occurrence of genetic recombination between them. Genetic recombination was especially common between genotypes I and J within Group 2. The data presented indicate a high genetic identity between Group 1 and Group 2 genotypes of *E. bieneusi*, which could be responsible for the broad host range and high zoonotic potential of Group 2 genotypes in China. As there is no effective treatment against *E. bieneusi*, the One Health approach should be used in the control and prevention of zoonotic transmission of the pathogen.

1. Introduction

Microsporidia are a diverse group of obligate intracellular fungi that cause microsporidiosis in a variety of vertebrates and invertebrates [1]. Among the >17 human-pathogenic species of microsporidia, *Enterocytozoon bieneusi* is the most common [2,3]. It causes diarrhea and other gastrointestinal symptoms in both immunocompetent and immunocompromised persons [4–8]. Among them, AIDS patients and children are especially susceptible to *E. bieneusi* [4,9–11]. In addition to the small intestine, biliary and pulmonary involvement has been observed in immunocompromised persons [12,13]. Foodborne, waterborne, and hospital-associated outbreaks of microsporidiosis by *E. bieneusi* have been reported [14–17]. *E. bieneusi* is also commonly detected in other mammals and birds, causing diarrhea in these animals [18–20]. The only drug effective against microsporidiosis caused by *E. bieneusi*, fumagillin, has high toxicity and is not commercially available in most countries [19,20].

DNA sequence analysis of the ribosomal internal transcribed spacer

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(*ITS*) is widely used in genetic characterizations of *E. bieneusi* isolates and assessment of infection sources in humans and public health potential of the pathogen from animals [21]. Phylogenetic analysis of the *ITS* sequences has led to the identification of at least 11 phylogenetic groups (Group 1–11) of genotypes [21]. Among them, Group 1 has over 300 genotypes, some of which have a broad host range, thus are zoonotic [2]. Group 2, in contrast, contains nearly 100 genotypes, and was considered a ruminant-specific group for a long time [22]. However, some of the common genotypes in Group 2 such as I, J, BEB4, and BEB6 have been recently found in humans and some other animals, suggesting that the host range of Group 2 could be broader than believed [18,21].

Some common genotypes in Group 2 appear to have geographical differences in host range. Most infections with Group 2 genotypes in humans and nonhuman primates were reported from China. A survey of children with diarrhea in Changchun identified some infections with Group 2 genotypes such as J, I, CHN2 and CHN3 [23]. Another report described the occurrence of the Group 2 genotype BEB6 (reported as SH5) in hospitalized children in Shanghai [17]. Both J and BEB6 were identified in children attending three hospitals in Zhengzhou [6]. In addition, several reports showed the occurrence of Group 2 genotypes I, J, BEB4, BEB6, BEB8, BSH, CD6, CM4, CM5, CM7, CM9, CM20, and CM21 in wild or captive nonhuman primates in China [24–28]. In contrast, there has been only one report of the Group 2 genotype BEB4 in children in other countries [29]. The difference in host range of some Group 2 genotypes from diverse areas could be an indication of the genetic uniqueness of Group 2 genotypes in China.

In addition to sequence analysis of the *ITS* locus, a multilocus sequence typing tool (MLST) targeting four other genetic loci is available for high-resolution typing of *E. bieneusi* [30]. It has been used in genetic characterizations of *E. bieneusi* isolates from humans and animals [27,31–37]. This has led to improved understanding of the population genetics and zoonotic potential of *E. bieneusi*, especially *ITS* genotypes in Group 1 [38].

The aim of the present study was to explore the population genetics and zoonotic potential of Group 2 genotypes of *E. bieneusi* using MLST characterization of several common *ITS* genotypes from various ruminants in China. The One Health implications of the findings are discussed.

2. Material and methods

2.1. Ethics statement

Fecal specimens from animals in this study were collected with the permission of the farm manager, and the animals were handled in accordance with the Animal Ethics Procedures and Guidelines of the People's Republic of China. The research protocol was reviewed and approved by the Ethics Committee of the South China Agricultural University.

2.2. Sample sources

A total of 121 Group 2 isolates of *E. bieneusi* from dairy cattle, beef cattle, yaks, Tibetan sheep, deer and golden takins in China were selected for the population genetic analysis (Fig. 1). They were from previous molecular epidemiological studies of *E. bieneusi* in animals [27,39–44]. The number of isolates and their *ITS* genotype designations are shown in Table 1. For comparative purposes, sequence data of 113

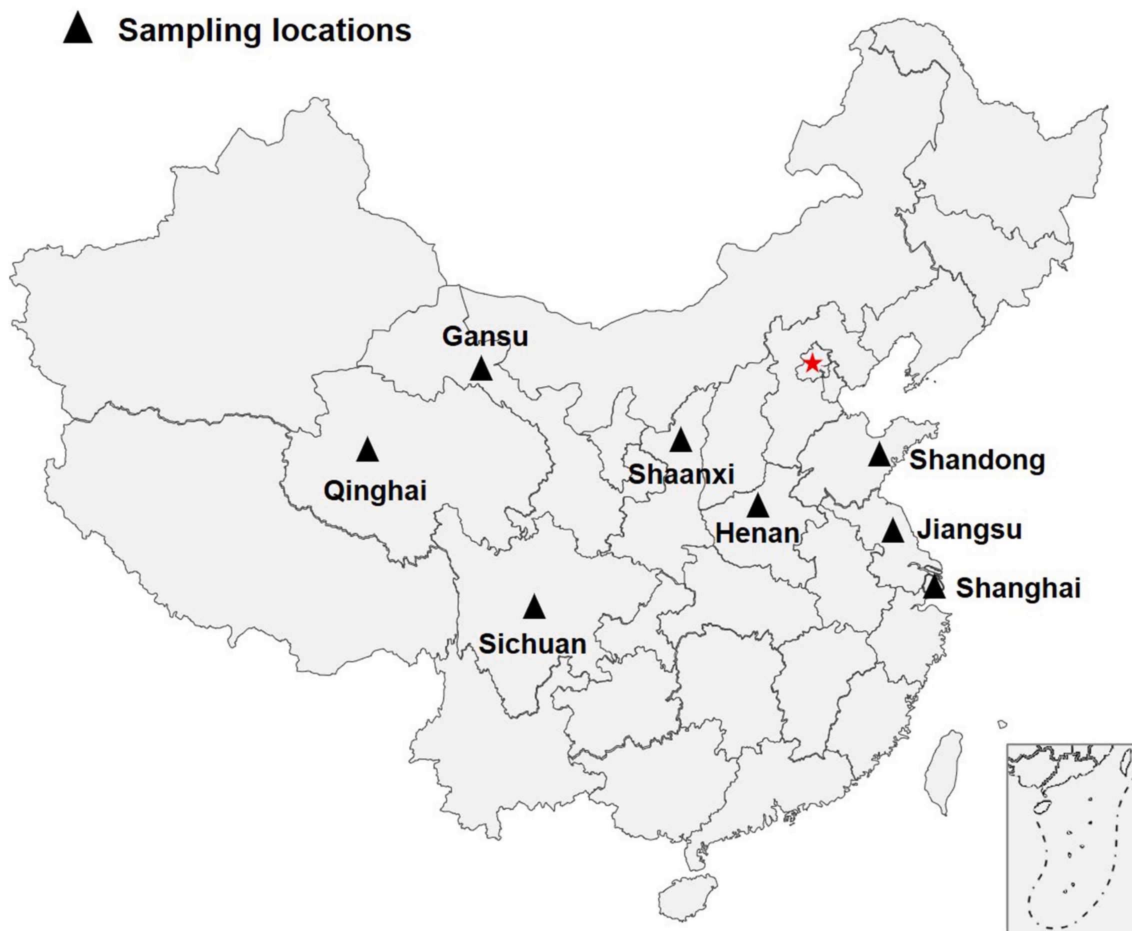

Fig. 1. Geographic locations of Group 2 isolates of *Enterocytozoon bieneusi* in this study.

Table 1
Enterocytozoon bieneusi isolates used in the study by geographic origin, host species and *ITS* genotype.

Groups	Location	No. of specimens	Host species	*ITS* genotypes	Reference
Group 1	/	113	NHP (113)	Type IV (30), CM1 (43), Peru8 (9), D (21), Henan V (10)	[10]
Group 2	Shanghai	33	Dairy cattle (33)	J (24), BEB4 (9)	[24]
	Jiangsu	27	Dairy cattle (27)	J (13), BEB4 (5), I (9)	[25]
	Henan	19	Dairy cattle (12)	J (3), BEB4 (2), I (7)	[23]
			Beef cattle (7)	J (2), I (5)	
	Shandong	3	Dairy cattle (3)	J (2), I (1)	
	Qinghai	29	Yak (10)	BEB4 (10)	
			Tibetan sheep (19)	BEB6 (19)	[27]
	Gansu	3	Tibetan sheep (3)	BEB6 (3)	[26]
	Sichuan	4	Deer (4)	BEB6 (4)	[11]
	Shaanxi	3	Golden takins (3)	BEB6 (3)	[28]
	Subtotal	121	Dairy cattle (75), Beef cattle (7), Yak (10), Tibetan sheep (21), Deer (4), Golden takins (3)	J (44), BEB4 (26), I (22), BEB6 (29)	
Total		234	Dairy cattle (75), Beef cattle (7), Yak (10), Tibetan sheep (21), Deer (4), Golden takins (3), NHP (113)	J (44), BEB4 (26), I (22), BEB6 (29), Type IV (30), CM1 (43), Peru8 (9), D (21), Henan V (10)	

Group 1 isolates of *E. bieneusi* (including *ITS* genotypes Type IV, CM1, D, Henan V and Peru8) from nonhuman primates in China were included in this analysis [26]. The sequences from five genetic loci in the latter were obtained from the associated investigators. MLST data from isolates from Tibetan sheep in Gansu, deer in Sichuan and golden takins in Shaanxi were from other published studies [27,42,44] and were downloaded from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/).

2.3. PCR analysis of MLST loci

The isolates used in the study were divided into different populations based on host origins, *ITS* genotypes and genogroups of *E. bieneusi*. They were analyzed at the MS1, MS3, MS4 and MS7 loci by PCR as described [30]. PCR amplification efficiency at each locus was calculated for each population. This was not done for the isolates from Tibetan sheep in Gansu and golden takins in Shaanxi, as the MLST data were obtained from the associated publications [42,44].

2.4. Population genetic analyses

The sequences of the five loci were concatenated in tandem for each isolate. The multilocus genotypes (MLGs) were analyzed for gene diversity (*Hd*), linkage disequilibrium (*LD*) and recombination events (*Rms*) using software DnaSP version 6.12.03 (http://www.ub.edu/dnasp/) with consideration of both sequence length polymorphism and nucleotide substitutions [45]. The population structure of *E. bieneusi* was further assessed by measuring the association of standard correlation index (I^S_A) and the relationship between V_D and *LE* using the online software LIAN version 3.7 (http://guanine.evolbio.mpg.de/cgi-bin/lian/lian.cgi.pl/query) [46].

2.5. Phylogenetic and sub-structure analyses

The maximum likelihood (ML) method implemented in MEGA 7 (www.megasoftware.net) [47] was used in a phylogenetic analysis of nucleotide sequences of all MLGs. The General Time Reversible model was used in the calculation of substitution rates, and bootstrap analysis with 1000 replicates was used to assess the reliability of cluster formation. STRUCTURE 2.3.4 (http://web.stanford.edu/group/pritchardlab/structure_software/release_versions/v2.3.4/html/structure.html) was used to assess population sub-structuring among all 234 isolates of *E. bieneusi*. The most appropriate K value was calculated using a statistics-based approach implemented in the online software Structure Harvester (http://taylor0.biology.ucla.edu/structureHarvester/). Additionally, principal coordinates analysis (PCoA) was performed on the generated matrices with the software GENALEX version 6.501 (http://biology-assets.anu.edu.au/GenAlEx) [48]. A median-joining phylogeny of MLST data was generated using Network software version 5.0 (www.fluxus-engineering.com/sharenet.htm) to estimate the genetic segregation and evolutionary trend of *E. bieneusi* [49].

2.6. Nucleotide sequence accession number

The partial nucleotide sequences of microsatellite and minisatellite loci used in this study have been deposited in the GenBank database under accession numbers (MS1: MT680817 - MT680845; MS3: MT680846 - MT680858; MS4: MT680859 - MT680869; and MS7: MT680870 - MT680877).

3. Results

3.1. PCR amplification efficiency of MLST loci

Data were available from 115 of the 121 Group 2 isolates for the calculation of the PCR amplification efficiency at the MLST loci. Altogether, the amplification efficiencies at the MS1, MS3, MS4, and MS7 loci were 86.1% (99/115), 72.2% (83/115), 82.6% (95/115), and 47.0% (54/115), respectively (Table 2). The amplification efficiency of Group 1 genotypes was similar among loci, ranging from 80.5% - 87.6%. Among Group 2 genotypes, only 4 of the 23 isolates of the *ITS* genotype BEB6 (4/23, 17.4%) were successfully amplified at the MS7 locus. In addition, isolates of other *ITS* genotypes in Group 2 also had low amplification efficiency at this locus. By host species, the amplification efficiencies of isolates from Tibetan sheep were 0% (0/19) and 5.3% (2/19) at the MS3 and MS7 loci, respectively, which were lower than those of other hosts.

3.2. Genetic structure of E. bieneusi

The concatenated sequences from five genetic loci were ~ 2174 bp in length. Fifty-three multilocus genotypes (MLGs, $Hd = 0.99$) were observed from the 121 Group 2 isolates. Of the 113 Group 1 isolates, 78 MLGs ($Hd = 0.98$) were identified. Tests for intragenic *LD* and recombination among segregating sites were performed on MLGs using DnaSP. Group 2 ($Y = 0.9927–0.0210X$) and Group 1 ($Y = 1.0061–0.2450X$) isolates both showed strong but incomplete linkage disequilibrium, suggesting the likely presence of genetic recombination in both groups. This was confirmed by recombination analysis; Group 2 and Group 1 had 5 and 7 recombination events (*Rms*), respectively.

Table 2
Amplification efficiency of *Enterocytozoon bieneusi* isolates at different genetic loci in China by *ITS* genotype and group and host species.[a]

Population	Group	Amplification efficiency (%)			
		MS1	MS3	MS4	MS7
ITS genotypes	J	90.9 (40/44)	79.5 (35/44)	86.4 (38/44)	43.2 (19/44)
	I	100.0 (22/22)	90.9 (20/22)	95.5 (21/22)	68.2 (15/22)
	BEB4	73.1 (19/26)	92.3 (24/26)	61.5 (16/26)	57.7 (15/26)
	BEB6	78.3 (18/23)	17.4 (4/23)	82.6 (19/23)	17.4 (4/23)
	CM1	76.7 (33/43)	90.7 (39/43)	76.7 (33/43)	79.1 (34/43)
	Type IV	83.3 (25/30)	86.7 (26/30)	80.0 (24/30)	80.0 (24/30)
	D	90.5 (19/21)	90.5 (19/21)	81.0 (17/21)	81.0 (17/21)
	Peru8	88.9 (8/9)	88.9 (8/9)	100.0 (9/9)	88.9 (8/9)
	Henan V	80.0 (8/10)	70.0 (7/10)	80.0 (8/10)	80.0 (8/10)
Animals	Dairy cattle	85.3 (64/75)	84.0 (63/75)	90.7 (68/75)	50.7 (38/75)
	Beef cattle	100.0 (7/7)	100.0 (7/7)	85.7 (6/7)	85.7 (6/7)
	Yak	100.0 (10/10)	90.0 (9/10)	10.0 (1/10)	50.0 (5/10)
	Tibetan sheep	78.9 (15/19)	0 (0/19)	84.2 (16/19)	5.3 (2/19)
	Deer	75.0 (3/4)	100.0 (4/4)	75.0 (3/4)	50.0 (2/4)
	NHP	82.3 (93/113)	87.6 (99/113)	80.5 (91/113)	80.5 (91/113)
Genotype groups	Group 1	82.3 (93/113)	87.6 (99/113)	80.5 (91/113)	80.5 (91/113)
	Group 2	86.1 (99/115)	72.2 (83/115)	82.6 (95/115)	47.0 (54/115)
Total		84.2 (192/228)	79.8 (182/228)	81.6 (186/228)	63.6 (145/228)

[a] Raw data from Group 1 genotypes were from a published study [10].

Table 3
Population genetics of *Enterocytozoon bieneusi* based on analysis of concatenated sequences from five genetic loci.[a]

Population		No. of isolates	*Hd*	*LD* (|*D'*|)	*Rms*
ITS genotypes	BEB6	8	1	Y = 0.9938–0.0073X	3
	BEB4	14	0.97	Y = 0.9883 + 0.0109X	1
	I	15	0.96	Y = 0.9648–0.5343X	1
	J	16	0.97	Y = 1.0274–0.1289X	1
	CM1	27	0.94	Y = 1.0492–0.5721X	4
	D	15	0.9	Y = 1.0601–0.6949X	1
	Type-IV	21	0.95	Y = 1.0063–0.1367X	2
	Henan V	7	0.71	NA	0
	Peru8	8	0.96	Y = 0.9853–0.1301X	3
Animals	Dairy cattle	34	0.99	Y = 0.9587–0.0443X	2
	Beef cattle	6	1	Y = 1.1622–0.4153X	1
	Tibetan sheep	3	1	Y = 1.0000 + 0.0000X	0
	Yak	5	0.9	NA	0
	Golden takins	3	1	Y = 1.0000 + 0.0000X	0
	Deer	2	1	Y = 1.0000 + 0.0000X	0
	NHP	78	0.98	Y = 1.0061–0.2450X	8
Groups	Group 1	78	0.98	Y = 1.0061–0.2450X	8
	Group 2	53	0.99	Y = 0.9927–0.0210X	5
Total		131	0.99	Y = 1.0046–0.1008X	14

Hd: gene diversity; *LD* (|D'|): linkage disequilibrium between sites; *Rms*: minimum number of recombination events.
[a] Raw data from Group 1 genotypes were from a published study [10].

The genetic characteristics (*Hd*, *LD* and *Rms*) of the populations by *ITS* genotype and host are presented in Table 3. Among them, isolates of most *ITS* genotypes showed high genetic diversity with the *Hd* values ranging from 0.9 to 1.0, except for Henan V isolates ($Hd = 0.71$). Several recombination events were detected in most *ITS* genotypes ($Rms = 1$–4), except for Henan V ($Rm = 0$). By host species, complete *LD* (Y = 1.0000 + 0.0000X) were observed in isolates from Tibetan sheep, golden takins and deer, with no recombination event being detected. In contrast, isolates from dairy and beef cattle had incomplete *LD* with some genetic recombination events (Table 3).

In the analysis of genetic structure with V_D and L measurements, similar to the Group 1 population, strong *LD* was obtained from the overall Group 2 population ($I^S_A = 0.2100$, $P_{MC} < 0.001$, and V_D: 1.4518 > L: 0.8876) (Table 4). By ITS genotype, however, genotype I, J and BEB6 all showed the absence of *LD* (genotypes I: $I^S_A = -0.0791$, $P_{MC} = 0.987$, and $V_D < L$; genotypes J: $I^S_A = 0.0110$, $P_{MC} = 0.370$, and $V_D < L$; genotypes BEB6: $I^S_A = 0.0070$, $P_{MC} = 0.268$, and $V_D < L$), indicating that the three subpopulations under comparison had relatively frequent genetic recombination (Table 4). In addition, most of the *E. bieneusi* subpopulations by host (beef cattle, yaks, Tibetan sheep and golden takins) also had genetic recombination, except for the subpopulation in dairy cattle (Table 4).

3.3. Population sub-structures

Maximum likelihood analysis of the sequences grouped the 94 MLGs of Groups 1 and 2 of *E. bieneusi* into two major evolutionary clusters (Fig. 2). Among them, one cluster contained most Group 1 isolates from NHPs and one Group 2 isolate of *ITS* genotype BEB6 from deer. The other cluster contained the remaining Group 2 isolates and several Group 1 isolates genotyped as Henan V and Peru8 at the *ITS* locus (Fig. 2). There was some genetic differentiation among *ITS* genotypes within Group 2, with most BEB4 and BEB6 isolates forming their own clusters (Fig. 2).

A similar result was obtained in STRUCTURE analysis of allelic data. At the optimal K = 7, isolates from Group 1 mostly formed two subpopulations, with Henan V forming one and the remaining isolates forming another. There was little admixture between the two subpopulations of Group 1 (Fig. 3). Within Group 2, isolates of *ITS* genotypes BEB4 and BEB6 mostly formed their own clusters with little genetic admixture between them. Isolates of *ITS* genotypes I and J, however, did not form any genotype-specific clusters, and there was significant genetic admixture between them (Fig. 3). This was largely supported by results of the PCoA analysis, in which isolates of Group 2 genotypes BEB4, I and J clustered together, although isolates of BEB6 mostly clustered with Group 1 isolates (Fig. 4). In the network analysis, within Group 2, isolates of BEB4 and BEB6 mostly formed their own clusters, while there was no clear differentiation of genotypes I and J (Fig. 5). In contrast, isolates of Group 1 genotypes mostly did not form any genotype-specific clusters, except for Henan V isolates, which formed a cluster just outside Group 2 (Fig. 5).

Table 4
Results of linkage disequilibrium analysis of *Enterocytozoon bieneusi* based on allelic profile data from five genetic loci.[a]

Population		No. of isolates	I^S_A	P_{MC}	V_D	L	$V_D > L$
ITS genotype	I	12	−0.0791	0.987	0.4159	0.8774	N
	J	14	0.011	0.37	0.537	0.6409	N
	BEB4	11	0.1908	0.016	1.5131	1.2909	Y
	BEB6	8	0.007	0.268	0.3003	0.4484	N
	D	9	−0.0133	0.787	0.5683	0.7968	N
	CM1	16	−0.0103	0.533	0.7661	1.0182	N
	Peru8	7	0.0357	0.385	0.7619	1.0619	N
	Type IV	13	0.0158	0.398	0.7126	0.8685	N
	Henan V	4	NA	1	NA	NA	NA
Host	Beef cattle	6	0.0264	0.54	0.9238	1.3524	N
	Dairy cattle	27	0.1011	< 0.001	1.1789	0.9732	Y
	Yak	4	−0.0423	1	0.3	0.7	N
	Tibetan sheep	3	−0.25	1	0.7411	3	N
	Deer	2	NA	< 0.001	NA	NA	NA
	Golden takins	3	0.125	1	0.3333	0.3333	N
	NHP	49	0.1091	< 0.001	1.322	1.0224	Y
Genotype group	Group 1	49	0.1091	< 0.001	1.322	1.0224	Y
	Group 2	45	0.21	< 0.001	1.4518	0.8876	Y
All		94	0.27	< 0.001	1.2623	0.6344	Y

I^S_A: standardized index of association calculated using the LIAN 3.5 program; P_{MC}: significance of obtaining this value in 1000 simulations using the Monte Carlo method; V_D: variance of pairwise differences; L: 95% critical value for V_D; $V_D > L$ indicates linkage disequilibrium.
[a] Raw data from Group 1 genotypes are from a previous publication [10].

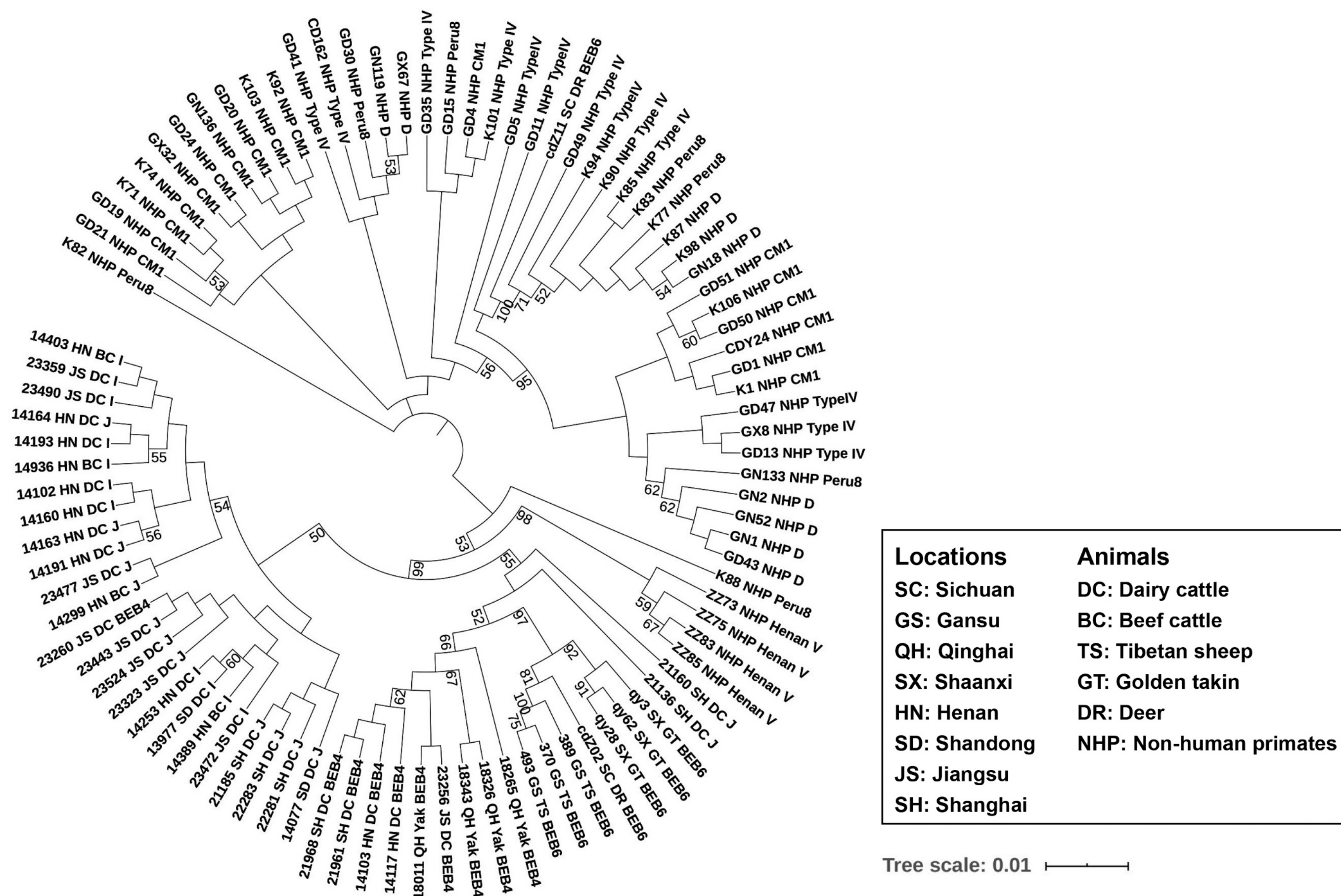

Fig. 2. Phylogenetic relationships among 94 MLGs of *Enterocytozoon bieneusi.* The phylogeny was inferred using maximum likelihood analysis of the concatenated nucleotide sequences based on substitution rates calculated using the General Time Reversible model. Bootstrap values above 50% from 1000 replicates are displayed on branches.

4. Discussion

The MLST data generated in the present study have shown high genetic similarity between Group 1 and Group 2 genotypes of *E. bieneusi* in China. Some of the *ITS* Group 2 genotypes, such as BEB6, clustered together with Group 1 genotypes in some of the population genetic analyses, while the opposite is also true for some Group 1 genotypes such as Henan V. While the two groups have significant sequence

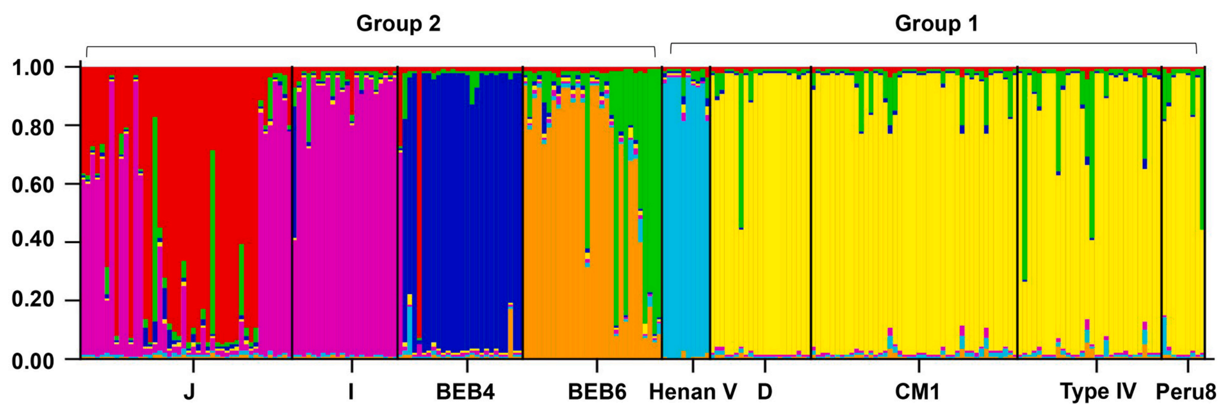

Fig. 3. Sub-structure of 234 *Enterocytozoon bieneusi* isolates inferred by Bayesian clustering of allelic data at the optimal K value of 7. The genogroup and *ITS* genotype identity of each isolate are shown above and below the figure, respectively.

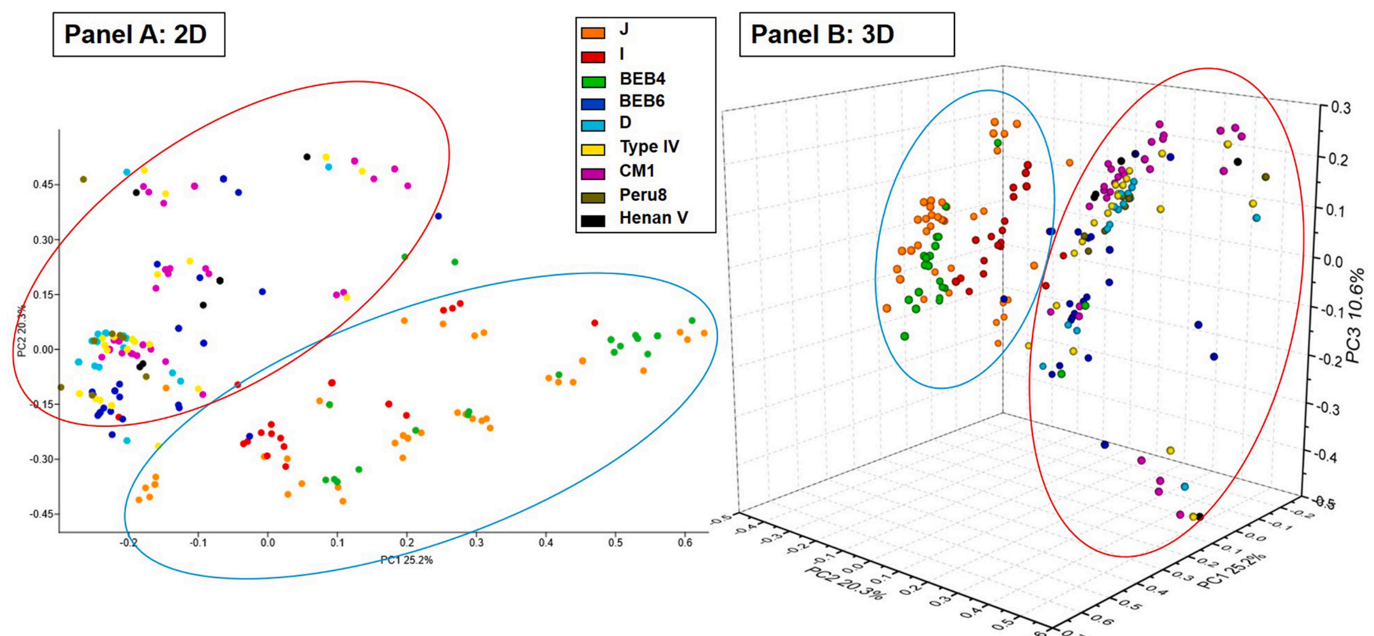

Fig. 4. Results of the principal coordinate analysis of 234 *Enterocytozoon bieneusi* isolates based on pairwise genetic distances (Panel A: 2D, Panel B: 3D). Each solid sphere represents an MLG. The color of the sphere represents the *ITS* genotype identity of isolates.

differences at some genetic loci, as reflected by differences in amplification efficiency between them at some genetic loci, there is no clear genetic differentiation in the overall genetic data between Groups 1 and 2. This could be the result of genetic recommendation among *E. bieneusi* isolates. Significant genetic admixtures were observed between Group 2 genotype BEB6 and Group 1 genotypes. Genetic recombination also exists between genotypes I and J within Group 2.

The occurrence of genetic admixture is partially attributable to the high genetic diversity within *E. bieneusi*. In addition to the presence of hundreds of *ITS* genotypes in 11 phylogenetic groups, results of the study suggest that there is a high genetic diversity within each of the *ITS* genotypes. The genetic diversity (*Hd*) of Group 2 isolates was 0.99, with the identification of 53 MLGs among the four *ITS* genotypes analyzed. Similar results (78 MLGs) were found among five Group 1 genotypes from nonhuman primates, which were used for comparison. This is in agreement with other MLST characterizations of *E. bieneusi* in China [34,36,37]. High genetic diversity is known to genetically select pathogens adapted to different hosts and environments, weakening the host specificity of eukaryotic pathogens [50].

The high prevalence of *E. bieneusi* and frequent concurrence of mixed *E. bieneusi* genotypes in farm animals have further facilitated the occurrence of genetic recombination. Epidemiological studies in China have shown that the mean prevalence of *E. bieneusi* infections is 5.7% (188/3271) in humans, 17.8% (948/5318) in nonhuman primates, 14.0% (783/5594) in cattle, 31.9% (978/3068) in goats and sheep, and 55.9% (1101/1969) in pigs [51]. Among them, concurrent infections with multiple *E. bieneusi* genotypes of Group 1 have been found in some hosts [52–55]. Similar situations have also occurred among Group 2 genotypes [41,56]. In addition, mixed infections of Group 1 and 2 genotypes have been observed in some animals, such as the concurrence of genotypes D and J, as well as Type IV and BEB4 in dairy cattle [40,57]. Co-infection of the same host by Group 1 and 2 of *E. bieneusi* can undoubtedly increase the genetic exchanges between genotypes. The introgression of Group 1 sequences into Group 2 probably has resulted in the host range expansion in Group 2 genotypes in China, making them infective to humans. Results of the *LD* analysis support the occurrence of genetic recombination in *E. bieneusi* in China. Genetic recombination appears to be especially common between *ITS* genotypes BEB6 and Group 1 genotypes. In addition, similar situation also occurs between Group 2 genotypes I and J. This is evident in the ML tree, STRUCRURE

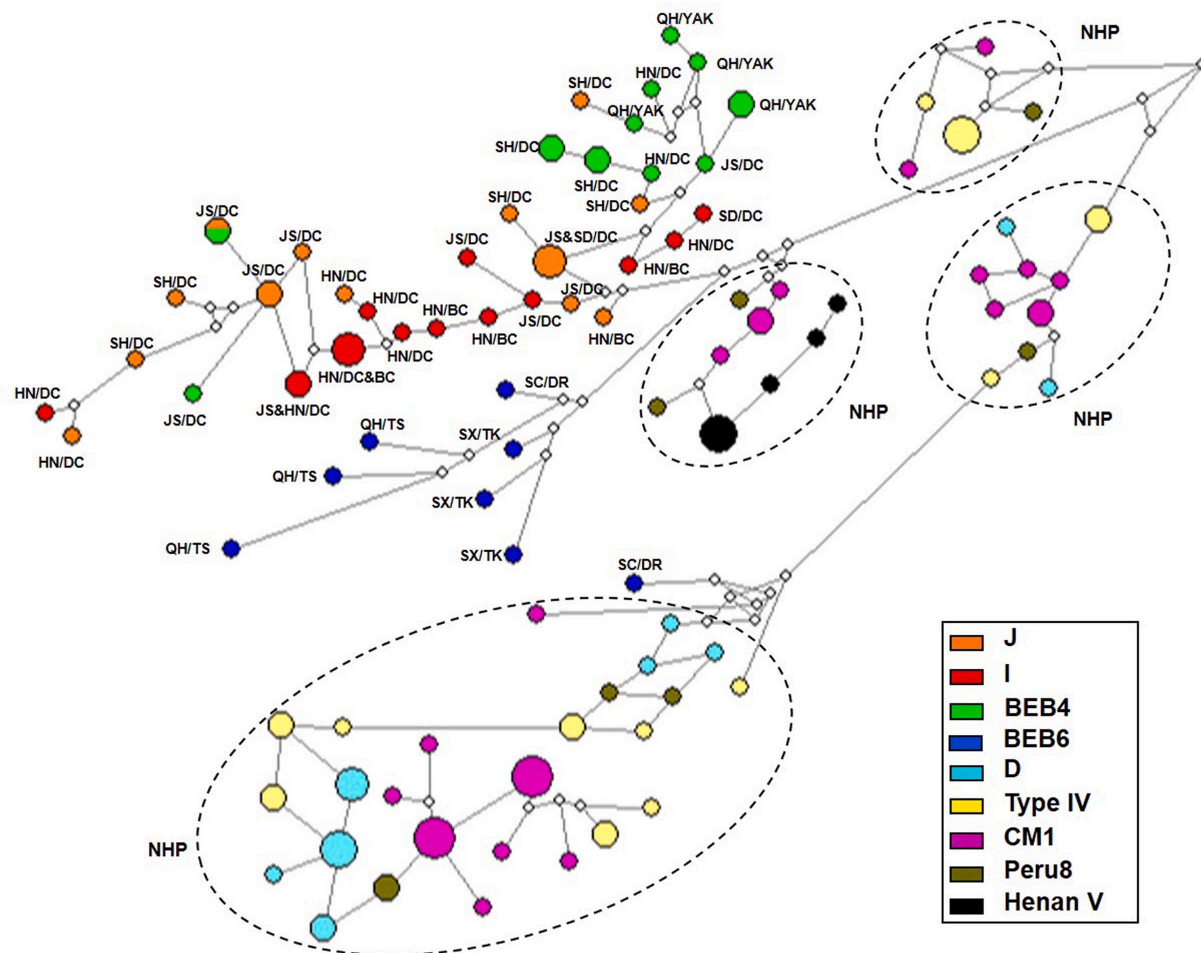

Fig. 5. Phylogeny of 131 *Enterocytozoon bieneusi* isolates inferred by median-joining network analysis of MLST data at five genetic loci. The size of each circle is proportional to the number of isolates with the MLG, while the color of the circles representing the *ITS* genotypes of the MLG.

plot, and PCoA and network graphs constructed using the MLST data, with some Group 2 isolates (especially BEB6) and Group 1 isolates clustering together. Results of the STRUCTURE and network analyses appear to suggest that the genetic recombination is mostly due to the mutual introgression of sequences from these genotypes. Therefore, genetic recombination contributes to the high genetic diversity of *E. bieneusi* in China.

The introgression of Group 1 sequences could have led to the broad host range and increased zoonotic potential of Group 2 *E. bieneusi* in China. Contradictory to the finding the Group 2 genotypes almost exclusively in ruminants elsewhere, Group 2 genotypes are increasingly reported in other animals in China, including horses, dogs, cats, rodents, nonhuman primates, and various wildlife and semi-domesticated animals [25–28,58–68]. Molecular epidemiological studies have further shown that Group 2 genotypes of *E. bieneusi* are more likely to infect humans in China than in other regions [21]. The broad host range of Group 2 genotypes in China could be attributed to their genetic similarity to Group 1 genotypes. The occurrence of genetic recombination in *E. bieneusi* is probably facilitated the habitat sharing among different species of animals in the traditional Chinese farming system, in which several animal species are frequently kept together on farm and pastures. The less stringent biosecurity practiced by many farms also increase the introduction of pathogens by rodents and small wild mammals.

One Health measures should be implemented to reduce the occurrence of zoonotic transmission of *E. bieneusi* in China. The genetic similarity between Group 1 and 2 genotypes suggests that ruminants could be an important source of human infection with *E. bieneusi* in China [56]. Ruminants are already known as important sources of several major enteric pathogens such as *Cryptosporidium parvum* and pathogenic *Escherichia coli* in humans [69]. As there is a lack of effective treatment against *E. bieneusi*, measures such as reducing contact with farm animals, adequate hygiene and hand washing should be implemented to prevent the acquisition of this zoonotic pathogen, as recommended for zoonotic enteric pathogens in general [70,71]. As introgression of foreign genetic elements is known to increase the pathogenicity and transmissibility of zoonotic enteric pathogens [72], these One Health measures will also likely reduce the emergence of highly pathogenic strains of *E. bieneusi* with broad host ranges.

5. Conclusions

High genetic diversity is apparently present in the most common Group 2 genotypes of *E. bieneusi*. This high genetic heterogeneity is partially attributable to the genetic recombination with Group 1 genotypes from diverse animals as well as among Group 2 genotypes from ruminants. The genetic recombination with Group 1 has apparently played a major role in shaping the population genetics of Group 2 genotypes, and is probably responsible for broadening the host range and human-infective potential of Group 2 of *E. bieneusi* in China. One Health measures focusing on the interruption of transmission cycle should be implemented to control microsporidiosis caused by zoonotic *E. bieneusi*.

Authors' contributions

Yaoyu Feng, Lihua Xiao: Conceptualization, Methodology, Funding acquisition, Writing- reviewing and editing, Supervision. Zhenjie Zhang: Data curation, Writing- original draft. Jingbo Ma, Xitong Huang, Xi Wen, Wen Jiang, Li Chen: Resources, Investigation. Yaqiong Guo, Na Li: Formal analysis, Software. Longxian Zhang: Investigation.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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