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Zoonotic *Cryptosporidium* Species and *Enterocytozoon bieneusi* Genotypes in HIV-Positive Patients on Antiretroviral Therapy

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Molecular diagnostic tools have been used increasingly in the characterization of the transmission of cryptosporidiosis and microsporidiosis in developing countries. However, few studies have examined the distribution of *Cryptosporidium* species and *Enterocytozoon bieneusi* genotypes in AIDS patients receiving antiretroviral therapy. In the present study, 683 HIV-positive patients in the National Free Antiretroviral Therapy Program in China and 683 matched HIV-negative controls were enrolled. *Cryptosporidium* species and subtypes and *Enterocytozoon bieneusi* genotypes were detected and differentiated by PCR and DNA sequencing. The infection rates were 1.5% and 0.15% for *Cryptosporidium* and 5.7% and 4.2% for *E. bieneusi* in HIV-positive and HIV-negative participants, respectively. The majority (8/11) of *Cryptosporidium* cases were infections by zoonotic species, including *Cryptosporidium meleagridis* (5), *Cryptosporidium parvum* (2), and *Cryptosporidium suis* (1). Prevalent *E. bieneusi* genotypes detected, including EbpC (39), D (12), and type IV (7), were also potentially zoonotic. The common occurrence of EbpC was a feature of *E. bieneusi* transmission not seen in other areas. Contact with animals was a risk factor for both cryptosporidiosis and microsporidiosis. The results suggest that zoonotic transmission was significant in the epidemiology of both diseases in rural AIDS patients in China.

Cryptosporidium spp. and microsporidia (especially *Enterocytozoon bieneusi*, the most common species infecting humans) are AIDS-defining pathogens (1). They induce chronic diarrhea, reduce life quality, and increase mortality in HIV-positive patients (2, 3). In developed countries, the morbidity and mortality caused by both pathogens have been reduced significantly by highly active antiretroviral therapy (HAART), introduced in 1996 (2, 3). However, HAART is still not widely available in developing countries. In most developing countries, including China, current HAART regimes do not include protease inhibitors, which appear to contribute significantly to the effect of HAART against cryptosporidiosis and microsporidiosis due to their effects on immune reconstitution (4). Thus, the effect of HAART on the occurrence of cryptosporidiosis and microsporidiosis in HIV-positive patients in developing countries remains unclear (5). Limited reports have shown that cryptosporidiosis and microsporidiosis are still common in HIV-positive patients receiving HAART in developing countries (6–8). Since there is no well-defined or reliable treatment for the diseases caused by *Cryptosporidium* spp. and *E. bieneusi* in immunocompromised persons, understanding their epidemiology is the key in the formulation of control strategies against cryptosporidiosis and microsporidiosis.

In recent years, molecular diagnostic tools have been widely used in the differentiation of *Cryptosporidium* species and subtypes and *E. bieneusi* genotypes and in the elucidation of transmission routes of cryptosporidiosis and microsporidiosis (9, 10). Data accumulated thus far suggest that anthroponotic transmission plays a more important role in the epidemiology of cryptosporidiosis in HIV-positive patients in developing countries and some industrialized nations such as the United States, but zoonotic transmission contributes significantly to the occurrence of cryptosporidiosis in HIV-positive patients in European countries (11, 12). In contrast, data on the distribution of *E. bieneusi* genotypes suggest that the transmission of *E. bieneusi* is mainly anthroponotic in developed countries (13–18) but both anthroponotic and zoonotic in developing countries (18–28). Differences in the transmission of the two diseases among countries and between rural and urban areas make studies of their epidemiology necessary.

Henan is the largest agricultural province in China and has been seriously affected by the HIV epidemic. Thousands of former plasma donors, almost all poor farmers who were paid to donate plasma by illegal plasma collectors in rural Henan, acquired HIV infection in the 1990s due to the unsanitary conditions during plasma collection. After the HIV outbreak, HIV began to spread via unprotected sexual contact and vertical transmission (29). By the end of 2006, 35,232 HIV-positive cases, with the majority (90.8%) of them being farmers, had been identified in 159 counties in Henan (29). In response to the large number of HIV-positive cases, the Chinese government initiated the National Free Antiretroviral Therapy Program (NFATP) in 2002, which provides free antiretroviral drugs to former plasma donors with HIV infection (30, 31). However, no data are available on the transmission of *Cryptosporidium* and *E. bieneusi* in HIV-positive persons in China, and few case-control studies have been conducted in developing countries to characterize the risk factors involved in the acquisition of the two opportunistic pathogens in HIV-positive patients (2, 3).

In this report, we describe the prevalence and genotype distri-

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TABLE 1 Demographics of the HIV-positive (case) and HIV-negative (control) groups in this study

Demographic variable	Case	Control[a]
Gender, no. (%)		
Male	329 (48.9)	312 (49.7)
Female	344 (51.1)	316 (50.3)
Age (yr)	4–72 (mean, 44.2; median, 45)	1–82 (mean, 41.3; median, 44)
Farming as occupation, no. (%)	628 (93.0)	384 (90.4)
Water supply source, no. (%)		
Tap water	37 (5.5)	1 (0.2)
Hand pump water	468 (69.2)	355 (83.7)
Well water	171 (25.3)	68 (16.1)
Family size, no. (%)		
≤6 members	640 (94.8)	410 (96.5)
>6 members	35 (5.2)	15 (3.5)
Presence of diarrhea, no. (%)		
Yes	263 (44.5)	127 (29.9)
No	328 (55.5)	298 (70.1)
Presence of animals in household, no. (%)		
Yes	425 (63.0)	267 (62.8)
No	250 (37.0)	158 (37.2)
$CD4^+$ cell count	372 (mean), 340 (median)	

[a] Only 425 participants provided information other than gender and age in this group.

bution of *Cryptosporidium* spp. and *E. bieneusi* in HIV-positive persons in NFATP in Henan, China, in a case-control study setting. Our data suggest that zoonotic transmission plays an important role in both cryptosporidiosis and microsporidiosis in HIV-positive patients on HAART.

MATERIALS AND METHODS

Study population. In March and April 2007 and November 2010, 860 and 506 patients in hospitals in Henan were recruited to participate in the study, respectively. Among them, half were HIV-positive patients enrolled in the NFATP (cases), and the other half were HIV-negative patients with similar demographic and socioeconomic backgrounds (controls). The demographic data of the two groups are shown in Table 1. Among them, 641 (49.3%) were males and 660 (50.7%) were females. The patients were 1 to 82 years in age with a median age of 44 years. Most (91.2%) of the participants were farmers and lived in rural areas. Demographic data, presence of diarrhea, recent $CD4^+$ cell counts, and potential risk factors related to food-borne, waterborne, person-to-person, and zoonotic transmission were collected from the participants by attending physicians using a structured questionnaire at the time of enrollment. Informed consent was obtained from all participants. No follow-up of participants was conducted. This study was approved by the institutional review board of the Henan Center for Disease Control and Prevention, China.

Stool specimen collection and processing. A single fecal specimen was collected from each case and control participant at the same time period and stored in 2.5% potassium dichromate solution at 4°C. Within several weeks of the two sampling rounds in 2007 and 2010, DNA was extracted from 200 μl of stool using the Fast DNA Spin kit for soil (Qbiogene, Irvine, CA), after the specimen was washed three times by centrifugation with deionized water. The DNA was extracted from the case and control pairs simultaneously and stored at −20°C before PCR analysis.

***Cryptosporidium* detection, genotyping, and subtyping.** An ~830-bp fragment of the small-subunit (SSU) rRNA gene was amplified by nested PCR as previously described (32). *Cryptosporidium* species were differentiated by restriction fragment length polymorphism (RFLP) analysis of the secondary PCR products using restriction enzymes SspI and VspI. To identify *Cryptosporidium hominis*, *Cryptosporidium parvum*, and *Cryptosporidium meleagridis* subtypes, an ~850-bp fragment of the 60-kDa glycoprotein (*gp60*) gene was amplified by nested PCR (33). The secondary PCR products were sequenced using the secondary PCR primers and an intermediary sequencing primer (5′-GAGATATATCTTGTT GCG-3′). The established subtype nomenclature was used to identify the *gp60* subtypes (34).

***Enterocytozoon bieneusi* detection and genotyping.** To detect *E. bieneusi*, an ~392-bp fragment of the rRNA gene, including the internal transcribed spacer (ITS), was amplified by nested PCR (28). Genotypes of *E. bieneusi* were determined by sequence analysis of the secondary PCR products and named according to the established nomenclature system (35).

DNA sequencing and data analysis. After being purified using Montage PCR filters (Millipore, Bedford, MA), the secondary PCR products were sequenced directly in both directions using an ABI BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and an ABI3130 genetic analyzer (Applied Biosystems). The nucleotide sequences of *Cryptosporidium* spp. and *E. bieneusi* were aligned with reference sequences downloaded from GenBank using ClustalX (http://www.clustal.org/; last accessed in November 2012) to determine their genotype or subtype identity. The *E. bieneusi* genotypes identified in the study were compared with known *E. bieneusi* genotypes using a neighbor-joining analysis of the aligned *E. bieneusi* sequences implemented in the program Mega 5 (http://www.megasoftware.net/; last accessed in November 2012). Bootstrap analysis with 1,000 replicates was used to assess the robustness of clusters.

Statistical analysis. A chi-square test or Fisher exact test was used to compare infection rates. The strength of association with risk factors was measured using the odds ratio (OR). Differences were considered significant at a P value of ≤0.05. All statistical analyses were performed using SPSS Statistics 17.0 (SPSS Inc., Chicago, IL).

Nucleotide sequence accession numbers. Unique nucleotide sequences generated from the study were deposited in GenBank under accession numbers JF691560 to JF691566 and JQ029720 to JQ029736.

RESULTS

Infection rates of *Cryptosporidium* and *E. bieneusi*. *Cryptosporidium* spp. were detected in 11 participants, including 10 (1.5%) HIV-positive patients and one (0.2%) HIV-negative patient. The difference in *Cryptosporidium* infection rates between HIV-positive and HIV-negative groups was significant ($P < 0.01$) (Table 2).

Enterocytozoon bieneusi was found in 68 study participants. Infection rates of *E. bieneusi* were 5.7% (39/683) and 4.2% (29/683) in HIV-positive and HIV-negative patients, respectively. The difference between the two groups was not significant ($P = 0.21$) (Table 2). Among the study patients, two were infected with both *Cryptosporidium* and *E. bieneusi*.

Species, genotypes, and subtypes of *Cryptosporidium* and *E. bieneusi*. Four *Cryptosporidium* species were found, including *C. meleagridis* (5 cases), *C. hominis* (3 cases), *C. parvum* (2 cases), and *C. suis* (1 case). Subtypes of *C. meleagridis* (IIIbA26G1R1, IIIbA27G1R1, IIIbA29G1R1, and IIIeA26G2R1), *C. hominis* (IbA19G2 and IeA12G3T3), and *C. parvum* (IIdA19G1) were determined for all but one *C. meleagridis* patient (Table 3).

Twelve *E. bieneusi* genotypes were found. The most prevalent genotypes were EbpC (39 cases), D (12 cases), and type IV (7 cases). Others included Peru8, Peru11, PigEBITS7, EbpD, and five

TABLE 2 Risk factors for cryptosporidiosis and microsporidiosis[c]

Risk factor	Specimen size, no. positive/total no.[a]	*Cryptosporidium* infection			*E. bieneusi* infection		
		No. positive (%)	OR (95% CI[d])	*P*	No. positive (%)	OR (95% CI[d])	*P*
Age (yr)							
0–9	70/1,342	1 (1.43)			6 (8.57)		
10–19	48/1,342	0			6 (12.50)		
20–29	30/1,342	1 (3.33)			0		
30–39	257/1,342	0		0.36	10 (3.89)		**0.05**
40–49	482/1,342	5 (1.04)			18 (3.73)		
50–59	364/1,342	4 (1.10)			23 (6.32)		
≥60	91/1,342	0			5 (5.49)		
Gender (male)	641/1,301	3 (0.47)	0.38 (0.10, 1.45)	0.14	34 (5.30)	1.03 (0.63, 1.68)	0.90
HIV infection	683/1,366	10 (1.46)	10.13 (1.29, 79.38)	**<0.01**	39 (5.71)	1.37 (0.84, 2.24)	0.21
Diarrhea in patients							
Case	263/651	3 (1.14)	0.74 (0.18, 2.96)	0.93	23 (8.75)	2.38 (4.66, 1.22)	**0.01**
Control	127/425				7 (5.51)	0.77 (0.32, 1.86)	0.56
CD4$^+$ cells (<200)[b]	158/590	3 (1.90)	1.65 (0.39, 7.00)	0.77	8 (5.06)	1.16 (0.50, 2.70)	0.73
Contact with patients with diarrhea	228/608	1 (0.44)	0.55 (0.06, 5.35)	1.00	14 (6.14)	0.89 (0.46, 1.74)	0.74
Animal in household							
Pig	253/1,100	5 (1.98)	3.40 (0.98, 11.82)	0.10	14 (5.53)	0.88 (0.48, 1.61)	0.67
Cattle	39/1,100	1 (2.56)	3.08 (0.38, 24.90)	0.80	4 (10.26)	1.81 (0.62, 5.25)	0.44
Dog	475/1,100	5 (1.05)	1.32 (0.38, 4.58)	0.91	28 (5.89)	0.94 (0.57, 1.55)	0.81
Cat	189/1,100	3 (1.59)	2.08 (0.53, 8.13)	0.51	9 (4.76)	0.74 (0.36, 1.51)	0.40
Sheep/goat	180/1,100	5 (2.78)	5.23 (1.50, 18.25)	**0.01**	11 (6.11)	1.00 (0.52, 1.96)	0.99
Chicken/duck	253/1,100	3 (1.19)	1.44 (0.37, 5.61)	0.88	18 (7.11)	1.25 (0.71, 2.18)	0.44
Water source							
Tap water	38/1,100	0		**0.01**	1 (2.63)		0.42
Hand pump water	823/1,100	4 (0.49)			48 (5.83)		
Well water	239/1,100	6 (2.51)			18 (7.53)		
Raw vegetable consumption	988/1,101	10 (1.01)		0.58	56 (5.67)	0.56 (0.28, 1.10)	0.09
Family of >6 members	50/1,101	0		1.00	7 (14.00)	2.64 (1.14, 6.12)	**0.04**
Hand washing	1,089/1,101	10 (0.92)		1.00	64 (5.88)	0.19 (0.05, 0.71)	**0.03**
Alcohol consumption	442/1,096	1 (0.23)	0.16 (0.02, 1.29)	0.10	23 (5.20)	0.78 (0.46, 1.31)	0.35

[a] Sample sizes differ for various factors due to missing patient information.
[b] Analysis conducted in the HIV-positive group only.
[c] Bold type for values indicates statistical significance.
[d] CI, confidence interval.

new genotypes named Henan-I to Henan-V and were found in one case each, mostly in the HIV-positive group (Table 3). These new genotypes were phylogenetically related to the genotype group 1, which contains most human-pathogenic *E. bieneusi* genotypes (Fig. 1). One patient with type IV and one with Peru8 were coinfected with *Cryptosporidium meleagridis*.

Risk factors for cryptosporidiosis and microsporidiosis. *Cryptosporidium* infection was significantly associated with HIV infection (10/683 in HIV-positive group versus 1/683 in HIV-negative group; $P < 0.01$) and use of well water as the drinking water supply (6/239 versus 4/861; $P = 0.01$). Patients with a history of animal contact in general had a higher occurrence of *Cryptosporidium* infection, but the association was significant only for sheep/goat keeping ($P = 0.01$) (Table 2).

A number of factors related to *E. bieneusi* infection were analyzed. An age of 10 to 19 years ($P = 0.04$) and living in a family with >6 members ($P = 0.04$) were associated with higher *E. bieneusi* prevalence, whereas hand washing before meals ($P = 0.03$) was a protective factor against *E. bieneusi* infection (Table 2).

A higher occurrence of diarrhea was observed in the HIV-positive group than in the HIV-negative group (263/651 versus 127/425; $P < 0.01$) (Table 1). In HIV-positive patients, patients with low CD4$^+$ cell counts (<200 cells/ml) were more likely to have diarrhea (74/154 versus 148/411; $P = 0.01$). However, we did not identify a significant association between CD4$^+$ cell count and *Cryptosporidium* or *E. bieneusi* infection (Table 2): the median CD4$^+$ cell count was 295 in 8 *Cryptosporidium*-positive patients and 339 in 582 *Cryptosporidium*-negative patients, whereas the median was 357 in 27 *E. bieneusi*-positive patients and 337 in 563 *E. bieneusi*-negative patients. Among HIV-positive patients, infection with *E. bieneusi* was significantly associated with the occurrence of diarrhea (Table 2).

Risk factors were also analyzed for prevalent *E. bieneusi* genotypes (Table 4). The most common, EbpC, was associated with

TABLE 3 *Cryptosporidium* and *E. bieneusi* genotypes/subtypes in HIV-positive and HIV-negative participants in Henan, China

Species	Subtype or genotype	No. of persons infected		Major host(s)[a]
		HIV$^+$	HIV$^-$	
C. hominis	IbA19G2	2		Humans
	IeA12G3T3		1	Humans
C. parvum	IIdA19G1	2		Humans, cattle, sheep, goats
C. meleagridis	IIIbA27G1R1	1		
	IIIbA29G1R1	1		
	IIIbA26G1R1	1		Chickens, turkeys, pet birds
	IIIeA26G2R1	1		
	Unknown	1		
C. suis		1		Pigs
	EbpC	18	21	Humans, pigs, wild mammals
	D	7	5	Humans, cattle, pigs, dogs, wild mammals
	IV	6	1	Humans, cats, cattle, dogs
	PigEBITS7	1		Pigs
E. bieneusi	EbpD	1		Pigs
	Peru8	1		Humans
	Peru11		1	Humans
	Henan-I	1		Humans (this study)
	Henan-II	1		Humans (this study)
	Henan-III	1		Humans (this study)
	Henan-IV	1		Humans (this study)
	Henan-V	1		Humans (this study)
	Unknown		1	

[a] Based on references 11, 12, and 35.

having pigs in the household ($P = 0.01$; Table 4). Genotype D was more prevalent in patients who were 0 to 19 years in age (6/12 versus 6/55; $P < 0.01$). The less common genotypes (those other than EbpC and D) were more often seen in the HIV-positive patients ($P < 0.01$) and patients with diarrhea ($P = 0.04$) (Table 4).

DISCUSSION

The low infection rates of *Cryptosporidium* (1.5%) and *E. bieneusi* (5.7%) in HIV-positive patients in this study could be mostly attributed to the NFATP. The infection rates were lower than those in studies conducted in other developing countries, where infection rates of 13.3 to 73.6% and 9.5 to 76.9% were reported for *Cryptosporidium* and microsporidia/*E. bieneusi*, respectively (7, 19, 22, 24, 26, 36–39). The infection rate of *Cryptosporidium* in this study was similar to that in HAART-receiving HIV-positive patients in Taiwan (1.2% in 332 patients) (40) and Denmark (1.0% in 96 patients) (41), as well as in Brazilian patients in the HAART era (0.3%) (42). A previous study also showed that infection rates of *Cryptosporidium* decreased significantly in patients after the initiation of HAART in Brazil: 8.1% in 482 pretreated patients and none in 100 posttreated patients (43). In a large cohort study in 10 European countries and Australia, the relative risk for contracting cryptosporidiosis was reduced by 96% in the HAART era (44). Therefore, even though protease inhibitors were not used, HAART in the NFATP was probably able to reduce the transmission of cryptosporidiosis. Previously, it was shown that NFATP was effective in reducing mortality in HIV-positive former plasma donors in China (31).

In agreement with the gradual decrease of *E. bieneusi* prevalence in HIV-positive patients receiving HAART in developed countries (17, 45), a significant decrease in *E. bieneusi* occurrence was observed in HIV-positive patients enrolled in 2010 compared with those enrolled in 2007 (8/253 versus 31/430; $P = 0.03$) in this study, suggesting that the NFATP might have played a role in this reduction, especially in cryptosporidiosis. In developing countries, the relationship between HAART and *E. bieneusi* occurrence in HIV-positive patients has seldom been examined. A gradual decrease in the prevalence of *E. bieneusi* was seen in a Thai orphanage (including 77 HIV-positive patients on HAART and 463 HIV-negative patients), although it was attributed to increased sanitation rather than antiretroviral therapy (25).

Zoonotic contact appeared to be an important risk factor in the acquisition of cryptosporidiosis in HIV-positive farmers in this study. Three of the four *Cryptosporidium* species detected, including *C. parvum*, *C. meleagridis*, and *C. suis*, were known parasites of animals. Although *C. meleagridis* was reported in six pediatric patients in China in one recent study (46), *C. parvum* and *C. suis* were reported in humans in China for the first time. The dominance of zoonotic *Cryptosporidium* spp. observed is different from most observations on cryptosporidiosis in humans in developing countries, where anthroponotic transmission plays a more important role in the epidemiology of cryptosporidiosis in HIV-positive patients (11, 12). Previously, it was thought that cryptosporidiosis transmission in China was also largely anthroponotic in nature (33, 46, 47). Differences in study areas (rural versus urban) could be responsible for the discrepancy in major transmission routes between current and earlier studies.

The occurrence of zoonotic cryptosporidiosis was supported by *C. parvum* subtyping. Both *C. parvum* cases were caused by the IIdA19G1 subtype, which was detected recently in calves in Henan (48). Although thus far they have not been found in sheep and goats in Henan (49), *C. parvum* IId subtypes are known to preferentially infect these animals rather than cattle (50, 51), a fact in agreement with the identification of sheep/goat keeping as a risk factor for cryptosporidiosis in this study. Another related *C. parvum* IId subtype, IIdA15G1, is common in rodents in Henan (52).

Consistent with the relatively highoccurrence of zoonotic *Cryptosporidium* species, 90% of *E. bieneusi* infections in the farmers were caused by genotypes commonly found in animals, including all three dominant genotypes (EbpC, D, and type IV), PigEBITS7, and EbpD. Only Peru8 and Peru11, which were found in one HIV-positive and HIV-negative patient each, have thus far been reported only in humans (35). The common occurrence of the EbpC genotype in HIV-positive patients in Henan differs from findings in other areas. Thus far, only 10 cases of infection by EbpC strains have been reported in HIV-positive patients in developing countries and none have been reported in industrialized nations. Previously, the zoonotic genotypes D (90 cases) and type IV (87 cases) and the anthroponotic genotype A (142 cases) were the most common genotypes of *E. bieneusi* in HIV-positive patients in developing countries (18–23, 25–28). In contrast, the anthroponotic genotype B was the dominant genotype in HIV-positive patients in Europe and Australia, infecting at least 164 HIV-positive patients, compared to only two patients infected by the zoonotic genotype D and 14 patients infected by type IV (13–18). Interestingly, the most common anthroponotic genotype in HIV-

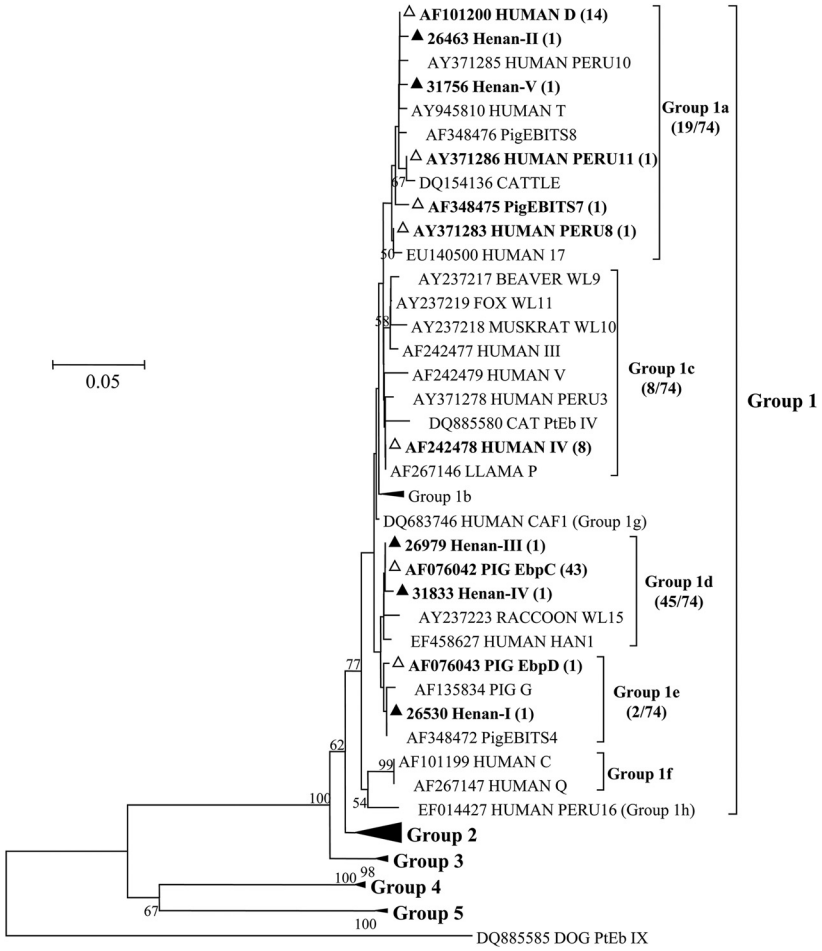

FIG 1 Phylogenetic relationship of *E. bieneusi* genotypes identified in this study and other genotypes previously deposited in GenBank as inferred by a neighbor-joining analysis of ITS sequences, based on the p-distance model. Percent bootstrap values greater than 50% from 1,000 replicates are shown to the left of nodes. Novel and known genotypes identified in this study are indicated by ▲ and △, respectively.

positive patients in developing countries, genotype A, was found in only three HIV-positive patients in Europe (15), and similarly, the dominant anthroponotic genotype in developed countries, genotype B, was found in only one HIV-positive patient in Africa (24).

The likely occurrence of zoonotic cryptosporidiosis and microsporidiosis in this study was also supported by risk factor analysis. In this study, contact with animals, especially sheep and goats, was associated with cryptosporidiosis. Likewise, for *E. bie-*

TABLE 4 Significant genotype-specific risk factors in 67 *E. bieneusi*-positive patients

Risk factor	Genotype	Sample size[a]	No. positive (%)[b]	OR (95% CI[c])	*P*[d]
Age of 0–19 yr	D	12	6 (50.00)	8.17 (1.99, 33.58)	**<0.01**
Female gender	Not EbpC or D	33	11 (33.33)	2.90 (0.88, 9.57)	0.07
HIV infection	Not EbpC or D	39	14 (35.90)	7.28 (1.50, 35.35)	**<0.01**
Diarrhea	Not EbpC or D	30	11 (36.67)	3.47 (1.04, 11.57)	**0.04**
Animal in household	EbpC				
Pig		14	12 (85.71)	6.23 (1.27, 30.58)	**0.01**
Cattle		4	2 (50.00)	0.75 (0.10, 5.67)	1.00
Dog		27	13 (48.15)	0.56 (0.21, 1.50)	0.24
Cat		9	4 (44.44)	0.57 (0.14, 2.32)	0.66
Sheep/goat		11	7 (63.64)	1.41 (0.37, 5.37)	0.86
Chicken/duck		18	12 (66.67)	1.77 (0.57, 5.47)	0.32

[a] Number out of 67 patients (65 patients for diarrhea) with the risk factor.
[b] Number of patients with the genotype.
[c] CI, confidence interval.
[d] Bold type for values indicates statistical significance.

neusi, contact with pigs was strongly associated with the most prevalent genotype EbpC, which was initially identified in pigs and is a common genotype in this species of animals (35). The zoonotic microsporidiosis transmission was likely caused by direct contact with infected animals or contaminated premises, as neither water nor eating raw vegetables was a risk factor. Correspondingly, hand washing before meals was a protective factor against microsporidiosis. Poor hygiene in general was probably a risk factor for microsporidiosis, as indicated also by the association of microsporidiosis with big families.

In conclusion, zoonotic *Cryptosporidium* species and *E. bieneusi* genotypes are still present in some HAART-receiving patients in rural China. Minimizing contact with animals and maintaining good hygiene practices should be advocated to reduce the transmission of *Cryptosporidium* spp. and *E. bieneusi* in HIV-positive rural patients in addition to the adherence to HAART regimes.

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