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Occurrence, Source, and Human Infection Potential of *Cryptosporidium* and *Enterocytozoon bieneusi* in Drinking Source Water in Shanghai, China, during a Pig Carcass Disposal Incident

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Abstract

In March 2013, thousands of domestic pig carcasses were found floating in the Huangpu River, a drinking source water in Shanghai, China. To investigate the impact of the pig carcass incident on microbial water quality, 178 river water samples were collected from the upper Huangpu River from March 2013 to March 2014. Samples were concentrated by calcium carbonate flocculation and examined for host-adapted *Cryptosporidium* and *Enterocytozoon bieneusi* by polymerase chain reaction (PCR). Positive PCR products were sequenced to determine *Cryptosporidium* species and *E. bieneusi* genotypes. A total of 67 (37.6%) and 56 (31.5%) samples were PCR-positive for *Cryptosporidium* and *E. bieneusi*, respectively. The occurrence rates of *Cryptosporidium* and *E. bieneusi* in March 2013 (83.3%; 41.7%) and May 2013 (73.5%; 44.1%) were significantly higher than rates in later sampling times. Among the 13 *Cryptosporidium* species/genotypes identified, *C. andersoni* and *C. suis* were the most common species, being found in 38 and 27 samples, respectively. Seventeen *E. bieneusi* genotypes were found, belonging to 11 established genotypes (EbpC, EbpA, D, CS-8, PtEb IX, Peru 8, Peru 11, PigEBITS4, EbpB, G, O) and six new ones (RWSH1 to RWSH6), most of which belonged to pig-adapted Groups 1d and 1e. EbpC was the most common genotype, being found in 37 samples. The distribution of *Cryptosporidium* species and *E. bieneusi* genotypes suggest that dead pigs contributed significantly to *Cryptosporidium* and *E. bieneusi* contamination in the Huangpu River. Although most *Cryptosporidium* species found in river water were not major human pathogens, the majority of *E. bieneusi* genotypes detected were endemic in China. Data from this study should be useful in the development of strategies in addressing future contamination events in drinking water supplies.

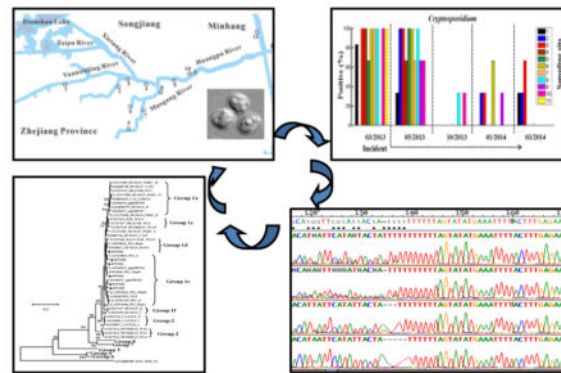
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Notes

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

The authors declare no competing financial interest.

Graphical abstract



INTRODUCTION

Cryptosporidium spp. and *Enterocytozoon bieneusi* are significant causes of diarrhea and enteric diseases (cryptosporidiosis and microsporidiosis) in humans and animals.^{1,2} The environmentally resistant oocysts (from *Cryptosporidium* spp.) and spores (from *E. bieneusi*) shed in feces have been frequently detected in water,^{3–5} and epidemiological and environmental studies have identified consuming contaminated water as an important risk factor for cryptosporidiosis and microsporidiosis in humans.^{6,7} Currently, complete removal and inactivation of *Cryptosporidium* oocysts and *E. bieneusi* spores are difficult to achieve during conventional water treatment.^{8,9} Therefore, these enteric pathogens represent a significant challenge to public health and drinking water authorities, especially in developing countries due to their common occurrence and insufficient removal during drinking water treatment.^{10,11} Like in many industrialized nations, *Cryptosporidium* is one of the two pathogens included in the Standard for Drinking Water Quality in China.¹² In addition, both *Cryptosporidium* and microsporidia are category B biodefense agents defined by the National Institutes of Health, USA.¹³

The standard method for the identification of *Cryptosporidium* oocysts in water is the United States Environmental Protection Agency Method 1622 and its equivalents in other countries,¹⁴ which provide a quantitative assessment of *Cryptosporidium* oocysts in water samples. However, this method cannot differentiate species of *Cryptosporidium*, thus cannot assess the public health significance of oocysts in water. Unlike Method 1622, PCR-based techniques can differentiate human-infective *Cryptosporidium* species from those that infect only animals. Likewise, phylogenetic analysis of ribosomal internal transcribed spacer (ITS) sequences has revealed the existence of host adaptation in *E. bieneusi* genotypes, with parasites from specific hosts forming different groups. These characteristics make it possible to assess the sources and human infection potential of pathogens in water using molecular diagnostic tools.^{5,15,16}

In March 2013, more than 16 000 pig carcasses that had been dumped in Jiaxing, Zhejiang Province reached Shanghai via the upper Huangpu River, which has long been utilized as a major drinking source water in Shanghai, China.¹⁷ Pigs are commonly infected with

Cryptosporidium suis and *Cryptosporidium scrofarum*, although several other *Cryptosporidium* species/genotypes such as *C. felis*, *C. hominis*, *C. meleagridis*, *C. muris*, *C. parvum*, *C. tyzzeri*, *C. andersoni*, *Cryptosporidium* sp. Eire w65.5, and *Cryptosporidium* rat genotype I are occasionally present.^{18–21} Likewise, the majority of *E. bieneusi* genotypes identified in pigs belong to subgroups 1d and 1e in Group 1, which appear to be pig-adapted. Nevertheless, both common *Cryptosporidium* species in pigs, *C. suis* and *C. scrofarum*, have been found in humans, and some of the *E. bieneusi* genotypes in pigs, such as EbpA, EbpC, EbpD, PigEBITS5, and PigEBITS7, are well-known human pathogens.^{22–26} Thus, the pig carcasses in the Huangpu River can potentially present a major public health problem despite the initial assurance to the general public on its minimum human health threat by various local authorities.

In this study, we aimed to (i) assess the contribution of the pig carcass incident to the contamination of *Cryptosporidium* and *E. bieneusi* in the Huangpu River and (ii) evaluate the human infection potential of *Cryptosporidium* and *E. bieneusi* during the environmental contamination.

MATERIALS AND METHODS

Sampling Sites

The Huangpu River has long been utilized as an important water source in Shanghai. It has been providing 30% of the drinking water for approximately 20% of Shanghai residents since a new reservoir with water from the Yangtze River was put in full use in 2011. The water in the Huangpu River comes from a river network (Figure 1). Among different upstream tributaries, the two main ones, Yuanxiejing and Maogang, were affected by pig carcasses most, and contribute approximately 25% and 15% of input into the Huangpu River. These two upstreams originate from Zhejiang Province and flow through agricultural areas (livestock and poultry farms) and suburbs of Shanghai. Eleven sampling sites were selected along the upper Huangpu River in this study, all affected by the pig carcass disposal incident. Eight of the sites were along the two tributaries (Sites 1–8), whereas three remaining sites were located along the main stream of the Huangpu River (Sites 9–11; Figure 1). Among the latter, Site 10 was near the water intake for one of the drinking water treatment plants in Shanghai, whereas Site 11 was downstream of the pig carcass disposal incident and within the Shanghai city proper. Carcass salvage, widely reported in news media, occurred mostly at Sites 4 and 8.

Sample Collection and Processing

A total of 178 river water samples were collected from the 11 sites at five time points (March, May, and October of 2013 and January and March of 2014). At each time, 36 water samples were collected, except for May 2013 when 34 samples were collected. Clean plastic containers (10 L) were used for collection of water approximately 20 cm below the surface near the edge of the river. Pathogens in the 10 L water samples were concentrated by calcium carbonate flocculation as previously described.²⁷ The concentrates were stored at –80 °C prior to DNA extraction.

DNA Extraction and Molecular Analysis

Genomic DNA was extracted from 0.5 mL of sample concentrates using the FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA) and eluted in 100 μ L of reagent-grade water as described previously.²⁸ DNA was stored at -20°C until being analyzed by nested PCR (see below) five times for each genetic target, using 2 μ L of the extraction DNA in PCR. The secondary PCR products were examined by 1.5% agarose gel electrophoresis. Positive PCR products were sequenced on an ABI 3130 sequencer (Applied Biosystems, Foster City, CA).

Cryptosporidium Detection and Genotyping

An approximately 587 bp fragment of the small-subunit (SSU) rRNA gene was amplified by nested PCR as previously described.²⁹ *Cryptosporidium* species present were differentiated by DNA sequence analysis. *Cryptosporidium parvum* and *C. hominis* in these samples were further subtyped by PCR and sequence analysis of an ~400 bp fragment of the 60 kDa glycoprotein (gp60).³⁰ A new PCR with better amplification efficiency for *C. meleagridis* was used in subtyping *C. meleagridis*, which amplified an ~955 bp fragment of the gp60 gene.³¹ The established subtype nomenclature was used to classify gp60 subtypes.

Detection and Genotyping of *E. bieneusi*

To detect *E. bieneusi*, an ~392 bp fragment of the ITS was amplified by nested PCR as previously described.³² Genotypes of *E. bieneusi* were determined by sequence analysis of the secondary PCR products and named according to the established nomenclature.³³ To assess the host source of the genotypes found, a neighbor-joining analysis of ITS sequences obtained was conducted using genetic distances calculated by the Kimura 2-parameter model implemented in Mega 6.0 (<http://www.megasoftware.net/>).

Statistical Analysis

Data were analyzed using SPSS 19.0 for Windows (SPSS Inc., Chicago, USA). The χ^2 test was used to compare the difference in pathogen occurrence among different sampling dates and sites. Differences were considered significant when $p < 0.05$.

Nucleotide Sequence Accession Numbers

Unique nucleotide sequences generated from this study were deposited in the GenBank under accession numbers KM496311–KM496316.

RESULTS

Occurrence of *Cryptosporidium* spp. and *E. bieneusi* in Raw Water

Of the 178 river water samples analyzed, 67 (37.6%) and 56 (31.5%) were PCR-positive for *Cryptosporidium* and *E. bieneusi*, respectively (Table 1). For *Cryptosporidium*, occurrence rates in March 2013 (30/36; 83.3%) and May 2013 (25/34; 73.5%) were significant higher ($p < 0.05$) than those in October 2013 (2/36; 5.5%), January 2014 (5/36; 13.9%) and March 2014 (5/36; 13.9%). The same trend was also observed in the occurrence of *E. bieneusi*; 15/36 (41.7%), 15/34 (44.1%), 6/36 (16.7%), 9/36 (25.0%) and 11/36 (30.5%) samples were positive in March 2013, May 2013, October 2013, January 2014, and March 2014,

respectively. The difference in *E. bieneusi* occurrence among five sampling time points, however, was insignificant ($p > 0.05$).

By site, nine of the 11 sites (except for Sites 2 and 9) were positive for *Cryptosporidium* in March 2013, all with occurrence rates $>66.6\%$. In May 2013, ten of the 11 sites (except for Site 11) were positive, mostly with occurrence rates $>66.6\%$. At other sampling time, only 2–4 sites were positive for *Cryptosporidium*, with much lower occurrence rates (Figure 2). For *E. bieneusi*, nine of the 11 sites (except for Sites 2 and 9) were positive in March 2013, whereas eight of the 11 sites (except for Sites 3, 9 and 10) were positive in May 2013. In contrast, only 2–6 sites were positive for *E. bieneusi* at other sampling time points (Figure 2). Overall, sites with higher *Cryptosporidium* occurrence also had higher *E. bieneusi* occurrence (25/72 or 34.7% versus 27/72 or 37.5% at four sites along the Yuanxiejing River; 28/62 or 45.2% versus 27/62 or 43.5% at four sites along the Maogang River; 3/44 or 6.8% versus 13/44 or 29.5% at three sites along the mainstream of the Huangpu River).

***Cryptosporidium* Species and Subtypes**

Altogether, 13 *Cryptosporidium* species/genotypes were found in river water samples, including *C. andersoni*, *C. suis*, *C. baileyi*, *C. scrofarum*, *C. meleagridis*, *C. parvum*, *C. hominis*, *C. ryanae*, *C. fragile*, *C. cuniculus*, rat genotype IV, avian genotype II, and avian genotype III. The most common species were *C. andersoni* and *C. suis*, being found in 38 and 27 water samples, respectively. *Cryptosporidium baileyi*, *C. scrofarum*, *C. meleagridis*, *C. parvum*, and *C. hominis* were found in 16, 8, 4, 3, and 2 water samples, respectively. The remaining *Cryptosporidium* species or genotypes each occurred in only a single water sample. By site, *C. andersoni* and *C. suis* were also the most common species, being found at all sites (Table 2). The frequent detection of the two species occurred mostly during and shortly after the pig carcass incident (Table 1). Afterward, they were only occasionally detected in water samples taken at the 11 sites.

Further subtyping of positive samples of *C. hominis*, *C. parvum*, *C. cuniculus*, and *C. meleagridis* at the gp60 locus identified IaA18R4 subtype of *C. hominis* (1), IIdA19G1 of *C. parvum* (1), VbA20 of *C. cuniculus* (1), and IIIbA24G1 of *C. meleagridis* (1). All the subtypes were found only during the pig carcass incident (March 2013).

***Enterocytozoon bieneusi* Genotypes**

A total of 17 *E. bieneusi* ITS genotypes were identified in this study, including 11 known ones (EbpC, EbpA, D, CS-8, PtEb IX, Peru 8, Peru 11, PigEBITS4, EbpB, G, and O) and six new ones (RWSH1 to RWSH6). EbpC was the most common genotype, being found in 37 samples. Genotypes EbpA, D, CS-8 and PtEb IX were found in 7, 7, 6, and 4 samples, respectively. The remaining genotypes, Peru 8, Peru 11, PigEBITS4, EbpB, G, and O, and six new genotypes were each observed in only one sample (Table 1). By site, EbpC was also the most common genotype (Table 2), being found at all sites except for one (Site 9 in the mainstream). The frequency of EbpC detection, however, was much lower in samples taken after the pig carcass incident (16/108 or 14.8%) than those taken during or shortly after the incident (21/70 or 30.0%; $p < 0.05$; Table 1).

Phylogenetic Relationship among *E. bieneusi* Genotypes

Neighbor-joining analysis of the ITS sequences revealed that most of the *E. bieneusi* genotypes found in this study belonged to zoonotic Group 1, except for the PtEb IX, which was divergent from all other *E. bieneusi* genotype groups and was used as an outgroup to root the neighbor-joining tree (Figure 3). Within Group 1, pigEBITS4, G, O and CS-8 and RWSH2 formed subgroup 1d together with EbpC, and the new genotypes (RWSH1, RWSH3, RWSH4, RWSH5, and RWSH6) formed subgroup 1e together with EbpA, EbpB, and EbpD. The remaining known genotypes, including D, Peru 8, and Peru 11, belonged to subgroup 1a (Figure 3).

DISCUSSION

In this study we examined the contamination of the upper Huangpu River by *Cryptosporidium* and *E. bieneusi* after the pig carcass disposal incident. We found that 37.6% of the 178 river water samples were positive for *Cryptosporidium* and 31.5% were positive for *E. bieneusi* over a 1 year study period. Although the overall occurrence of *Cryptosporidium* in this study is similar to the one (28%) in a previous study of the Huangpu River water in Shanghai,³⁴ occurrence rates of *Cryptosporidium* varied significantly among the five sampling time points ($p < 0.05$), with the highest rate (83.3%) being observed in March 2013. Likewise, the occurrence of *E. bieneusi* was also the highest during the pig carcass incident (March 2013, 41.7%) and shortly after (May 2013, 44.1%). These rates were significantly higher than occurrence rates of *Cryptosporidium* spp. and *E. bieneusi* in river water in most other areas.^{3-5,35,36} The frequent detection of both parasites concurred with the pig carcasses incident which took place during March 2013. Carcasses of thousands of pigs were discarded upstream in Jiaxing (60 miles southwest of Shanghai) and were found floating in the Huangpu River, probably because the relaxed control of the disposal of dead pigs. The cleanup operation was complete at the end of the month. *Cryptosporidium* and *E. bieneusi* from the dead pigs might have led to the high occurrence of both parasites in the Huangpu River during the event, despite the assurance of minimum impacts on water quality by the local water utility (<http://www.popsci.com/science/article/2013-03/can-you-really-still-drink-water-shanghai>). *Cryptosporidium* and *E. bieneusi* occurrence was still maintained at a relatively high level two months after the pig carcass scandal (73.5% and 44.1% in May 2013, respectively). The pathogen occurrence decreased significantly subsequently to rates more compatible to rivers in other areas,^{3-5,35,36} as stricter policy on pig carcass disposal was implemented by Jiaxing and other local authorities upstream of the Huangpu River.

Molecular identification demonstrates that the majority of the *Cryptosporidium* found in river water samples appear to be derived from farm animals, especially pigs and cattle. In this study, *C. andersoni* was one of the dominant species, as demonstrated previously in a study conducted in the same area.³⁴ Similar to this study, a predominance of *C. andersoni* was also found in the Three Gorges Reservoir in China,³⁷ and in other studies in industrialized nations.³⁸⁻⁴² *Cryptosporidium andersoni* is the most common *Cryptosporidium* sp. in adult cattle in China.⁴³ Several other common bovine *Cryptosporidium* spp. were absent or detected only occasionally in this study, such as *C.*

parvum in calves less than 4 weeks of age, and *C. ryanae* and *C. bovis* in older calves. Thus, the data from this study confirm that adult cattle are important contamination sources for *Cryptosporidium* sp.

The second most commonly detected *Cryptosporidium* species in the study area, especially during the pig carcass incident, was *C. suis*, which is a dominant species in pigs. The high level of detection suggested that pigs were an important contamination source in the study area. This is supported by the common occurrence of another porcine *Cryptosporidium* species, *C. scrofarum*, in eight river samples. It is known that *C. suis* shedding continues for a longer duration than *C. scrofarum* shedding, and older pigs frequently have mixed infection of both parasites.^{18,44,45} Although both porcine species were identified in a small number of river water samples in southern-eastern China,⁴⁶ the high occurrence of them in this study indicated that the floating pig carcasses likely contributed significantly to *Cryptosporidium* contamination in March and May 2013. Previously, it was shown that both *C. suis* and *C. scrofarum* were prevalent in pigs from the Yangtze River Delta, China, including the region in this study.^{47–49} The continued presence of *C. andersoni*, *C. suis*, and *Cryptosporidium* species from birds (*C. baileyi*, *C. meleagridis*, and avian genotype III) after the pig carcass incident suggests that cattle, pig and poultry farms are major contributors to background *Cryptosporidium* contamination in the Huangpu River, despite the fact that the drainage basin is heavily populated by humans.

Results of *E. bieneusi* genotyping support the role of the pig carcass incident in pathogen contamination in the Huangpu River. Most of the known and all novel *E. bieneusi* genotypes in the Huangpu River belong to genotype Groups 1d and 1e, except for genotypes D, Peru 8, and Peru 11, which belong to Group 1a, and PtEb IX, which belongs to the outlier of common *E. bieneusi* genotype groups (Figure 3). The latter were only found in 7, 1, 1, and 4 samples, respectively. Groups 1d and 1e genotypes are known to preferentially infect pigs.^{26,50–53} The most common genotype in this study, EbpC, is the most prevalent genotype identified in pigs in China,^{26,54} and has also been widely reported in domestic pigs in Japan, Germany, Czech Republic, and Switzerland.^{51,55} Surprisingly, Group 2 *E. bieneusi* genotypes, which are almost exclusively found in ruminants, are absent in the Huangpu River water samples. This is possibly because Group 2 genotypes are shed at low intensity, thus are not often detected in surface water, as reported recently in a study of microsporidia in surface water in Spain.³⁶

Despite the assurance of minimum public health impact of the floating pig carcasses by the local authorities in the general news media, most of the pig-derived *E. bieneusi* genotypes are known human pathogens in China. For example, the dominant *E. bieneusi* genotype in water samples during the pig carcass incident, EbpC, is also the dominant *E. bieneusi* genotype in HIV-positive and HIV-negative adults in rural areas in Henan⁵⁴ and has been found in children^{56,57} and several nonhuman primate species in China.^{58,59} Several other *E. bieneusi* genotypes found in water samples in this study, such as D, EbpA, EbpD, Peru 8, and Peru 11, have also been found in humans in China.^{54,56} Although these *E. bieneusi* genotypes can infect humans, the low prevalence of *C. hominis* in river water samples in this study indicated that humans were not a major source for *E. bieneusi* contamination during the pig carcass incident.

In contrast, the common finding of *C. suis* and *C. scrofarum* in the Huangpu River water samples during the pig carcass incident might only represent a modest public health threat. Although both *Cryptosporidium* species are known human pathogens, they have been found in only a few human cases.^{54,60–62} This is also the case with the bovine-originated *C. andersoni*, which has also been found in only a few human cases.^{61,63–65} In a recent study, *C. andersoni* was identified as the dominant *Cryptosporidium* species in diarrheic patients in Shanghai,⁶⁶ but the validity of this observation has been questioned.⁶⁷ Some other less common *Cryptosporidium* species in water samples in this study, such as *C. hominis*, *C. parvum*, and *C. meleagridis*, are more significant human-pathogenic *Cryptosporidium* spp.¹

In conclusion, an increased occurrence of *Cryptosporidium* and *E. bieneusi* has been detected in the Huangpu River after the pig carcass incident, and results of *Cryptosporidium* and *E. bieneusi* genotyping support the dominant porcine-origin of both pathogens. As the dominant *E. bieneusi* genotype detected is a common human pathogen in China, the public health significance of the floating pig carcasses probably has been underestimated. Thus, further studies are needed to assess the occurrence of other human pathogens after the incident, allowing a full evaluation of its public health and environmental impacts. Only clear regulations and strict control of disposal of dead animals can prevent future occurrences of environmental contamination affecting human health.

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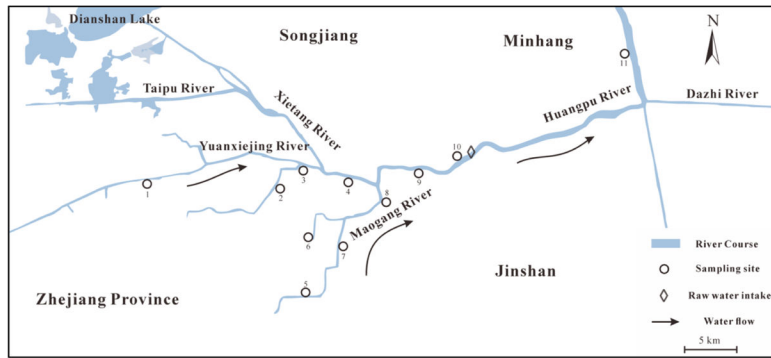


Figure 1. Sampling sites in the upper Huangpu River in Shanghai, China.

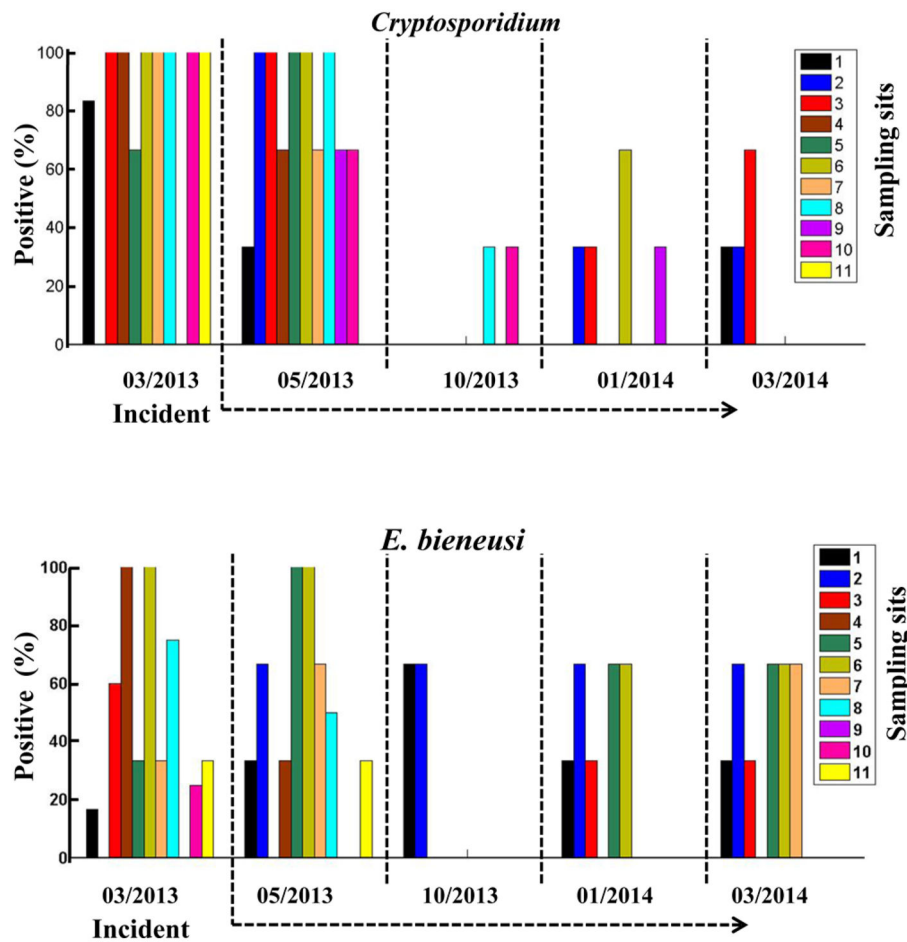


Figure 2. Spatial distribution of *Cryptosporidium* and *Enterocytozoon bienersi* at different locations of the Huangpu River since the pig carcass disposal incident. The percentage of positive samples is shown on the y axis.

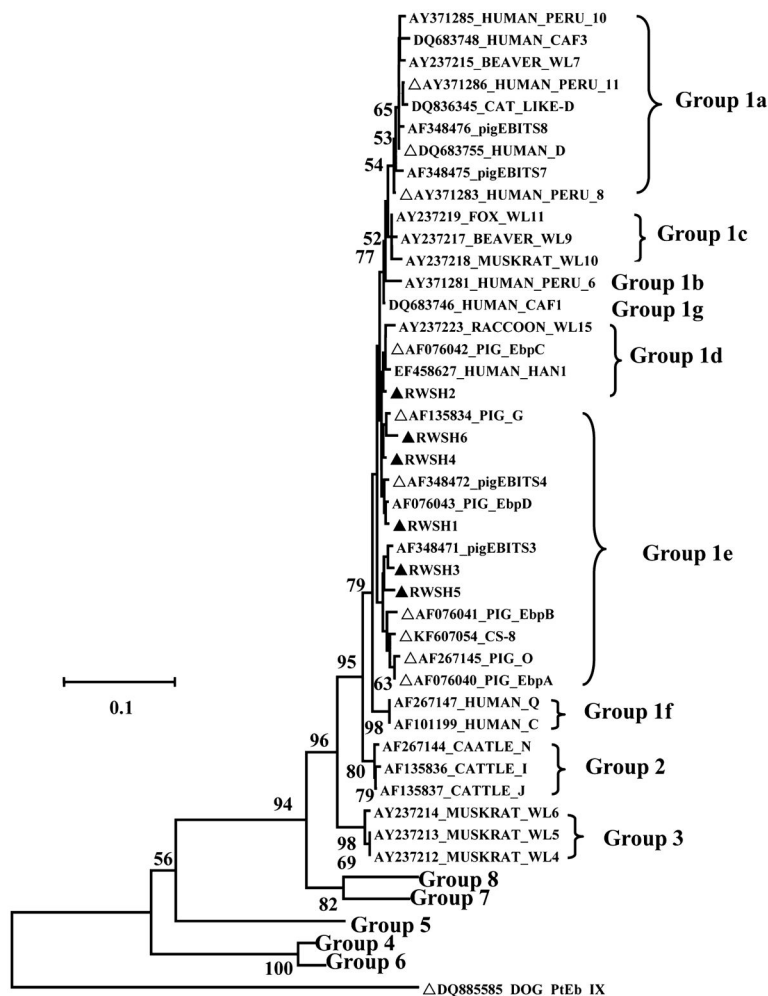


Figure 3. Phylogenetic relationship of *Enterocytozoon bieneusi* genotypes identified in this study and other genotypes previously deposited in GenBank as inferred by a neighbor-joining analysis of ITS sequences based on genetic distances calculated by the Kimura 2-parameter model. Bootstrap values greater than 50% from 1000 replicates are shown on nodes. Genotypes with “ Δ ” are known genotypes found in raw water samples; the novel genotypes in this study are indicated by “ \blacktriangle ”.

Table 1

Occurrence and Molecular Characterization of *Cryptosporidium* spp. and *Enterocytozoon bienersi* in the Upper Huangpu River in Shanghai, China

sampling time	no. of samples	<i>Cryptosporidium</i>		<i>Enterocytozoon bienersi</i>	
		no. of positive (%)	species (no. of positive sample)	no. of positive (%)	genotypes (no. of positive sample)
03/2013	36	30 (83.3)	<i>C. andersoni</i> (19), <i>C. suis</i> (16), <i>C. baileyi</i> (7), <i>C. scrofarum</i> (5), <i>C. hominis</i> (2), <i>C. parvum</i> (2), <i>C. meleagridis</i> (1), <i>C. ryanae</i> (1), <i>C. cuniculus</i> (1)	15 (41.7)	EbpC (11), EbpA (3), CS-8 (2), D (2), Peru 11 (1)
05/2013	34	25 (73.5)	<i>C. andersoni</i> (13), <i>C. suis</i> (7), <i>C. baileyi</i> (7), <i>C. scrofarum</i> (3), <i>C. meleagridis</i> (1), <i>C. parvum</i> (1), avian genotype II (1), rat genotype IV (1)	15 (44.1)	EbpC (10), D (4), PigEBITS4 (1), CS-8 (1), EbpA (1), EbpB (1), Peru 8 (1), G (1), RWSH1 (1)
10/2013	36	2 (5.5)	<i>C. suis</i> (1), <i>C. andersoni</i> (1), <i>C. baileyi</i> (1), <i>C. fragile</i> (1)	6 (16.7)	PEb IX (4), EbpC (2), CS-8 (1), O (1)
01/2014	36	5 (13.9)	<i>C. andersoni</i> (3), <i>C. suis</i> (1), <i>C. baileyi</i> (1), avian genotype III (1)	9 (25.0)	EbpC (6), CS-8 (2), EbpA (1), RWSH2 (1), RWSH3 (1), RWSH4 (1), RWSH5 (1)
03/2014	36	5 (13.9)	<i>C. andersoni</i> (2), <i>C. meleagridis</i> (2), <i>C. suis</i> (2)	11 (30.5)	EbpC (8), EbpA (2), D (1), RWSH6 (1)
Total	178	67 (37.6)	<i>C. andersoni</i> (38), <i>C. suis</i> (27), <i>C. baileyi</i> (16), <i>C. scrofarum</i> (8), <i>C. meleagridis</i> (4), <i>C. parvum</i> (3), <i>C. hominis</i> (2), <i>C. ryanae</i> (1), <i>C. cuniculus</i> (1), <i>C. fragile</i> (1), rat genotype IV (1), avian genotype II (1), avian genotype III (1)	56 (31.5)	EbpC (37), EbpA (7), D (7), CS-8 (6), PEb IX (4), Peru 8 (1), Peru 11 (1), PigEBITS4 (1), EbpB (1), G (1), O (1), RWSH1 (1), RWSH2 (1), RWSH3 (1), RWSH4 (1), RWSH5 (1), RWSH6 (1)

Table 2
Enterocytozoon bienersi and *Cryptosporidium* spp. at Different Locations along the Upper Huangpu River

sampling site Yuanxiejing (tributary 1)	no. of samples	<i>Enterocytozoon bienersi</i>			<i>Cryptosporidium</i>		
		genotypes (no. of positive sample)	major known host	species and/or genotype(s) (no. of positive sample)	major known host	possible major sources for pathogens in water	
1	27	EbpC (6), CS-8 (3), PEb IX (3), O (1), EbpA (1)	pigs, humans, dogs	<i>C. andersoni</i> (3), <i>C. suis</i> (3), <i>C. baileyi</i> (3), <i>C. meleagridis</i> (2), <i>C. hominis</i> (1), <i>C. ryanae</i> (1), <i>C. scrofarum</i> (1)	cattle, pigs, birds, humans	pigs, cattle, humans	
2	15	EbpC (3), EbpA (2), Peru8 (1), G (1), EbpB (1), PEb IX (1), RWSH3 (1)		<i>C. andersoni</i> (4), <i>C. baileyi</i> (1), <i>C. parvum</i> (1)			
3	17	EbpC (3), D (1), CS-8 (1), RWSH2 (1)		<i>C. suis</i> (5), <i>C. andersoni</i> (2), <i>C. baileyi</i> (2), <i>C. meleagridis</i> (2), <i>C. scrofarum</i> (1), rat genotype IV (1), avian genotype III (1), <i>C. cuniculus</i> (1), <i>C. hominis</i> (1)			
4 Maogang (tributary 2)	13	EbpC (2), PigEBITS4 (1), D (1)		<i>C. andersoni</i> (3), <i>C. suis</i> (1)			
5	15	EbpC (8), EbpA (1)	pigs, humans	<i>C. scrofarum</i> (5), <i>C. baileyi</i> (3), <i>C. andersoni</i> (2)	cattle, pigs, birds	pigs, cattle	
6	15	EbpC (7), D (1), EbpA (1), RWSH4 (1), RWSH5 (1), RWSH6 (1)		<i>C. andersoni</i> (6), <i>C. baileyi</i> (3), <i>C. suis</i> (2), avian genotype II (1)			
7	15	EbpC (4), D (2)		<i>C. suis</i> (5), <i>C. andersoni</i> (3)			
8 Huangpu River (mainstream)	17	EbpC (2), CS-8 (2), RWSH1 (1)		<i>C. andersoni</i> (7), <i>C. suis</i> (3), <i>C. baileyi</i> (3), <i>C. parvum</i> (1), <i>C. fragile</i> (1)			
9	13	not detected	pigs, humans	<i>C. andersoni</i> (2), <i>C. suis</i> (1)	pigs, cattle	pigs, cattle	
10	16	EbpC (1), D (1)		<i>C. suis</i> (6), <i>C. andersoni</i> (4)			
11	15	EbpC (1), EbpA (1), Peru11 (1), D (1)		<i>C. andersoni</i> (2), <i>C. suis</i> (1), <i>C. baileyi</i> (1), <i>C. parvum</i> (1)			