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BRIEF REPORT

Symptomatic *Entamoeba dispar* Infections Among Men Who Have Sex With Men, New York City, 2018

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Entamoeba histolytica is considered the primary species causing the parasitic gastrointestinal infection amebiasis. A cluster of amebiasis infections was identified in 2018 among men who have sex with men in New York City and was likely caused by *Entamoeba dispar*, traditionally considered to be nonpathogenic.

Keywords. amebiasis; *Entamoeba dispar*; *Entamoeba histolytica*; men who have sex with men.

Amebiasis is a parasitic diarrheal infection transmitted through ingestion of contaminated food or water or through oral/anal sexual contact. For decades, amebiasis has been documented as a sexually transmissible enteric infection (STEI) among men who have sex with men (MSM) in New York City (NYC) [1]. MSM status and sexual behavior such as oral/anal contact are known risk factors for *Entamoeba histolytica* [2]. *E histolytica* is considered the pathogenic species causing amebiasis, whereas the morphologically indistinguishable species *E dispar* was historically considered nonpathogenic [3].

The New York State Department of Health's Wadsworth Center developed a real-time polymerase chain reaction (PCR) assay to distinguish the 2 *Entamoeba* species. In 2018, an investigation into routinely reported amebiasis cases in NYC demonstrated that >80% were positive for *E dispar* and negative for *E histolytica* when tested by real-time PCR. The following report describes a small cluster of diarrheal illness related to *E dispar* among MSM in NYC.

METHODS

In NYC, all laboratory-confirmed amebiasis cases are reportable to the Department of Health and Mental Hygiene (DOHMH). A case of confirmed amebiasis is defined as demonstration of *E histolytica*/*E dispar* cysts or trophozoites in stool.

All reported amebiasis specimens are routinely requested to be submitted to the Wadsworth Center Parasitology Laboratory (WCPL) for confirmation. DNA is purified from submitted stool specimens via an automated DNA extractor (Qiagen). Real-time PCR to distinguish *E histolytica* and *E dispar* is performed by targeting small subunit ribosomal RNA with the primers and probes listed in Table 1. If a stained microscopy slide is provided, it is analyzed for the presence of *E histolytica*/*E dispar* and other parasites. Real-time PCR results for this study were confirmed via Sanger sequencing.

For specimens in the present cluster, DNA from stool specimens previously identified as *E dispar* positive were tested for the presence of bacterial pathogens. Real-time PCR assays specific for *Shigella* spp, *Salmonella* spp [4], *Campylobacter coli*, *C jejuni*, *C fetus*, *Vibrio alginolyticus*, *V cholerae*, *V mimicus*, *V parahaemolyticus*, *V vulnificus*, and *Escherichia coli* O157, as well as Shiga toxin 1 and Shiga toxin 2 [5], were performed with the primers and probes listed in Table 1.

Patients with amebiasis are not routinely interviewed by the DOHMH. However, spatiotemporal clusters of reportable communicable diseases, including amebiasis, are identified at the DOHMH through daily use of prospective space-time permutation scan statistics (SaTScan) [6]. Once the present cluster was identified, patients were interviewed by telephone to obtain clinical and risk behavior information. If the patient was not available for interview, the health care provider was interviewed. Patients/providers were asked about symptoms and medical history, travel history, and sexual behavior within a 2-week incubation period prior to symptom onset.

RESULTS

On 27 February 2018, a cluster of 7 cases of amebiasis was identified in a Manhattan neighborhood through a SaTScan signal. All patients presented with diarrheal illness and were diagnosed with amebiasis over a period of 11 days. No patient serology results were reported to the DOHMH, and no patients reported symptoms consistent with extraintestinal infection. All patients had *Entamoeba* cysts/trophozoites identified in stool by microscopy (ova and parasite examination, trichrome stain) at a

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Table 1. Primers and Probes Used to Perform the Real-time Polymerase Chain Reaction Assays

Organism	Sequence (5′ → 3′)	Target	Reference
Entamoeba histolytica	F: GCGGACGGCTCATTATAACA R: ATTGTCGTGGCATCCTAACTCA P: FAM/TCATTGAAT/ZEN/GAATTGGCCATTT/3IABkFQ	SSU rRNA	[12]
Entamoeba dispar	F: GCGGACGGCTCATTATAACA R: ATTGTCGTGGCATCCTAACTCA P: Cy5/TTACTTACATAAATTGGCCACTTTG/3BHQ_2	SSU rRNA	[12, 13]
Shigella spp	F: CGCTGCATGGCTGGAAA R: CAGCAGCAACAGCGAAAGAC P: FAM/CTCAGTGCCTCTGCGGAGCTTCGA/TAMRA	*ipaH*	This study
Salmonella spp	F: GCGTCGTACTATTGAAAAGCTGTCT R: TCACCATTAGTACCAGAATCAGTAATTC R: TCACCATTAGTACCAGGATCAGTAGTTC P: FAM/ACCTATAGTGCTGCTTTCTCTACTTAACAGTGCTCGTTT/BHQ1	*invA*	[4]
Campylobacter jejuni	F: TGCTAGTGAGGTTGCAAAAGAATT R: TCATTTCGCAAAAAAATCCAAA P: FAM/ACGATGATTAAATTCACAATTTTTTTCGCCAAA/TAMRA	*hipO*	[14]
Campylobacter coli	F: CATATTGTAAAACCAAAGCTTATCGG R: AGTCCAGCAATGTGTGCAATG P-VIC/TAAGCTCCAACTTCATCCGCAATCTCTCTAAATTT/TAMRA	*glyA*	[14]
Campylobacter fetus	F: TGCATCTCTCTCGCCGAAAT R: GTGCTGCAAGCCGTAGATAAAA P: TexRD/ACCAGCTGACCGGCATTTAGCACG/BHQ2	*sapD*	This study
Vibrio alginolyticus	F: GTTGTGAGAGCGGCACTCAA R: AAGACCAACCA AGGTGCCAAT P: JOE/TATTTCGCGCCTTTGGCGACGT/BHQ1	*pomA*	This study
Vibrio cholerae	F: GGATTGCTCTTCYCCATATGC R: CGTTACCAAAA GCCAAGTCGTAT P: CY5/CGGGTTTGCTGGCCCTGAAAGA/BHQ1	*pgm*	This study
V cholerae toxin	F: TCCAGCAGCAGATGGTTATGG R: CTCTTCCCTCCAAGCTCTATGC P: FAM-TTGGCAGGTTTCCCTCCG/BHQ1	*toxR*	This study
Vibrio mimicus	F: CGCTGTTCCCACAGGTCAAC R: TAACACTTCAAACGCAGCAACTC P: TAMN/TAGCGGTGTCACGCCTTCTTCAGTCA/BHQ2	*sodB*	This study
Vibrio parahaemolyticus	F: CGTTTGACGGACGCAGGT R: CGCACGAATTTTGTCGATCTC P: FAM/AGATGCGACGAAAGCGCCTCAGTTTAA/BHQ	*tlh*	This study
Vibrio vulnificus	F: GGTGTTAACTCGTAACGAACTGCAT R: TCTTTGAGCATTTTGCGTAAGGT P: CY5/AGTCGATGATTCCAGTCTAACTCAAGCGATTTCT/BHQ1	*toxR*	This study
Escherichia coli O157	F: AAAGGCATGTA GATTCGGTTGAA R: GGATCAACAGCAACCATGACA P: ROXN/CCTGGTTTCACACCTGGAGCATCCTGT/3BHQ_2	*rfb*	[5]
Shiga toxin-producing *E coli*	F: ATTCTGGGAAGCGTGGCATTA Fa: ATTCTGGGTAGCGTGGCATTA R: CGGGCACATAGAAGGAACTC A Ra: CGGACACATAGAAGGAAACTCAT Rb: GGGCACATAGAAGGAAGCTCAT P: VIC/TCATCATGCATCGCGAGTTGCCAG/TAMRA Pa: VIC/TCATCATGCATCACGAGTTGCCAGAA/TAMRA	*stx1*	[5]
	F: CAACGGACAGCAGTTATACCACTCT Fa: CAGCAGTTATACCACGCTGCAA R: AACGCCAGATATGATGAAACCA P: FAM/TTCCGGAATGCAAATCAGTCGTCACTC3BHQ_1	*stx2*	[5]

Abbreviation: SSU rRNA, small subunit ribosomal RNA.

Table 2. Characteristics of Patients With Amebiasis in a Cluster of Infections Among MSM, New York City, 2018

Patient	Age Group, y	Initial Laboratory Test	Confirmation Laboratory Test	Other Organisms Identified	Travel [a]	Symptoms
1	30–40	Trichrome stain	*Entamoeba dispar* real-time PCR, *Entamoeba histolytica* real-time PCR, ova and parasite, trichrome stain	*Blastocystis hominis*	Mexico	Diarrhea, fatigue, loss of appetite, nausea, vomiting
2	40–50	Trichrome stain, ova and parasite		*B hominis, Entamoeba hartmanni*	No	Abdominal pain, bloating, diarrhea (bloody and watery), fever, gas, nausea
3	40–50	Trichrome stain, ova and parasite		*B hominis, Giardia duodenalis*	No	Abdominal pain, bloating, diarrhea, fever, gas, loss of appetite, vomiting, weight loss
4 [b]	20–30	Trichrome stain		*B hominis, E hartmanni, Shigella* spp	No	Abdominal pain, bloating, diarrhea, fatigue, gas, weight loss
5	40–50	Trichrome stain, ova and parasite		*Entamoeba coli*	Mexico	Abdominal pain, bloating, diarrhea, fatigue, gas, loss of appetite, vomiting, weight loss
6	30–40	Ova and parasite	*E dispar* real-time PCR, *E histolytica* real-time PCR	*B hominis*	Italy	Bloating, diarrhea, fatigue, gas, nausea, weight loss
7	40–50	Trichrome stain	None	…	Unk	Abdominal pain/cramps, diarrhea

Abbreviations: MSM, men who have sex with men; PCR, polymerase chain reaction.

[a]International travel in 2 weeks prior to symptom onset.

[b]Patient had giardiasis infection diagnosed 30 days prior to *E dispar* diagnosis.

hospital or commercial laboratory (Table 2). Specimens from 6 patients were sent to the WCPL for confirmation. One specimen was mistakenly discarded by the commercial laboratory and was not able to be tested by the WCPL. Of the 6 specimens that were evaluated at the WCPL, all were positive by microscopy for *Entamoeba* cysts/trophozoites as well as for *E dispar* by real-time PCR. None of the samples were positive for *E histolytica* by real-time PCR. Of these 6 samples sent to the WCPL, 5 (83%) were also positive for *Blastocystis hominis*; 2 (33%) were positive for *Entamoeba hartmanni*; and 1 patient each was positive for *Shigella* spp, *Giardia duodenalis*, and *Entamoeba coli*. None of the 6 samples were positive for *Salmonella* spp, *C coli*, *C jejuni*, *C fetus*, *E coli* O157, *V alginolyticus*, *V cholerae*, *V mimicus*, *V parahaemolyticus*, *V vulnificus*, or Shiga toxin–producing *E coli* DNA.

All 7 patients lived within a 3-km radius of one another in a neighborhood in Upper Manhattan; 3 patients also worked in the neighborhood. Symptom onset dates ranged from June 2017 to February 2018. The median number of days between symptom onset and diagnosis was 41 (range, 4–245). Six patients were successfully interviewed; information from the seventh patient was collected from the health care provider. The median age was 41 years (range, 25–49); 86% (6/7) of patients identified as non-Hispanic White; and all identified as MSM. Two patients reported traveling to Mexico and 1 to Italy during the 2 weeks prior to symptom onset.

Of the 6 patients with known exposure history, 5 (83%) had sex in the 2 weeks prior to symptom onset, 4 (67%) had insertive anal sex, 2 (33%) inserted a finger into the partner's anus, and 2 (33%) had oral/anal contact (anilingus). Of the 5 patients who had sex in the 2 weeks prior to symptom onset, 4 (80%) inserted a penis, finger, or tongue in the partner's anus. Four patients denied having sex with someone met specifically through the internet in the 2 weeks before symptoms; 1 patient did not respond to this question. Whether any of these 7 patients had contact with each other was not ascertained.

All 7 patients were symptomatic, with diarrhea (100%), abdominal pain (86%), bloating (71%), and nausea (71%) most commonly reported. Five (71%) patients were treated with an antimicrobial or antiparasitic agent, most commonly metronidazole (60%). Through the match with the Bureau of Sexually Transmitted Infections and the Bureau of HIV, 1 patient was determined to be HIV positive (CD4 count not available) and had been previously diagnosed with syphilis. An additional patient had been diagnosed twice with chlamydia 3 years prior to his amebiasis infection.

DISCUSSION

Through advanced surveillance cluster detection analyses as well as in-house *Entamoeba* species–specific PCR assays, we were able to identify a cluster of patients with symptomatic *E dispar* amebiasis among MSM in NYC. All 7 patients were ill enough to seek care and exhibited significant gastrointestinal symptoms. Additionally, a majority of patients engaged in sexual behaviors that increase the likelihood of fecal/oral transmission prior to symptom onset.

A thorough laboratory evaluation suggests that coinfections with additional known enteric pathogens were uncommon in our cluster. One patient was coinfected with *Shigella* spp and

another with *G duodenalis*, both known STEIs among MSM [7]. However, the other organisms identified among the patients (*B hominis*, *E hartmanni*, and *E coli*) are not traditionally considered pathogenic.

Three patients reported international travel during the 2 weeks prior to symptom onset, including 2 to the endemic country of Mexico, so it is possible that these individuals were not infected in NYC. However, the fact that the patients who traveled were non-Hispanic White MSM diagnosed with *E dispar* close in space and time to other non-Hispanic White MSM with *E dispar* suggests that there might be an epidemiologic link in NYC. Parasitic STEIs such as amebiasis and giardiasis have long been documented among the MSM community in NYC [1], although amebiasis was traditionally attributed to *E histolytica* given the historical lack of diagnostic specificity. The ability to match *E dispar* or *E histolytica* isolates by molecular techniques would aid in better defining the epidemiology of this parasite.

E histolytica has historically been considered the pathogenic species causing amebiasis, in part because of the rare but severe clinical manifestations, including fulminating dysentery and liver abscess. Although *E dispar* has been considered nonpathogenic, several lines of evidence challenge this view. *E dispar* has been identified in patients with intestinal amebiasis in Brazil [8] and Italy [9], and patients in Brazil have also been diagnosed with amebic liver abscesses [8]. Strains of *E dispar* isolated from symptomatic and asymptomatic patients in South America phagocytized red blood cells and caused liver damage in hamsters, albeit to differing severity depending on the strain [10]. It should be noted that coinfections with pathogenic bacteria affect amoebic virulence factors [10, 11], although only 1 case of coinfection was detected among the cases reported here. It is also worth noting that the cycle threshold value for 5 patients was <25.0, indicating a significant parasite load.

Limitations of the present analysis include the small sample size and potential for misclassification in exposure recall for patients with symptom onsets significantly preceding their diagnosis dates. Nonetheless, the identification of a likely cluster of symptomatic sexually transmitted *E dispar* infections among MSM in NYC merits future work examining the pathogenicity of *E dispar*. Additionally, development of appropriate prevention options for amebiasis and other STEIs among MSM is warranted.

Notes

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Patient consent statement. This study does not include factors necessitating patient consent as specimens were tested for public health benefit.

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References

1. Phillips SC, Mildvan D, William DC, Gelb AM, White MC. Sexual transmission of enteric protozoa and helminths in a venereal-disease-clinic population. N Engl J Med **1981**; 305:603–6.
2. Hung CC, Wu PY, Chang SY, et al. Amebiasis among persons who sought voluntary counseling and testing for human immunodeficiency virus infection: a case-control study. Am J Trop Med Hyg **2011**; 84:65–9.
3. Heymann D. Control of communicable diseases manual. 19th ed. Washington, DC: American Public Health Association, **2008**.
4. Shushe O, Wroblewski D, MacGowan CE, Passaretti T, Musser K, Mingle L. Detection of *Salmonella* spp in retail meat products: a comparison between a discontinued commercial kit and a laboratory-developed screening method. Lett Appl Microbiol **2019**; 69:116–20.
5. Mingle LA, Garcia DL, Root TP, et al. Enhanced identification and characterization of non-O157 Shiga toxin–producing *Escherichia coli*: a six-year study. Foodborne Pathog Dis **2012**; 9:1028–36.
6. Greene SK, Peterson ER, Kapell D, Fine AD, Kulldorff M. Daily reportable disease spatiotemporal cluster detection, New York City, New York, USA, 2014–2015. Emerg Infect Dis **2016**; 22(10):1808–12.
7. Mitchell H, Hughes G. Recent epidemiology of sexually transmissible enteric infections in men who have sex with men. Curr Opin Infect Dis **2018**; 31:50–6.
8. Ximénez C, Cerritos R, Rojas L, et al. Human amebiasis: breaking the paradigm? Int J Environ Res Public Health **2010**; 7:1105–20.
9. Graffeo R, Archibusacci CM, Soldini S, Romano L, Masucci L. *Entamoeba dispar*: a rare case of enteritis in a patient living in a nonendemic area. Case Rep Gastrointest Med **2014**; 2014:498058.
10. da Silva CAV, de Oliveira IMC, Cruz RE, et al. South American *Entamoeba dispar* strains produce amoebic liver abscesses with different pathogenicities and evolutionary kinetics. Acta Trop **2021**; 224:106114.
11. Oliveira FMS, Fernandes ACDC, Sandes SHDC, et al. Co-infection by *Salmonella enterica* subsp *enterica* serovar typhimurium and *Entamoeba dispar* pathogenic strains enhances colitis and the expression of amoebic virulence factors. Microb Pathog **2021**; 158:105010.
12. Verweij JJ, Blangé RA, Templeton K, et al. Simultaneous detection of *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* in fecal samples by using multiplex real-time PCR. J Clin Microbiol **2004**; 42:1220–3.
13. Qvarnstrom Y, James C, Xayavong M, et al. Comparison of real-time PCR protocols for differential laboratory diagnosis of amebiasis. J Clin Microbiol **2005**; 43: 5491–7.
14. LaGier MJ, Joseph LA, Passaretti TV, Musser KA, Cirino NM. A real-time multiplexed PCR assay for rapid detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli*. Mol Cell Probes **2004**; 18:275–82.