Amebiasis among Persons Who Sought Voluntary Counseling and Testing for Human Immunodeficiency Virus Infection: A Case-Control Study

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Abstract. This case-control study aimed to characterize the factors associated with amebiasis, defined as presence of anti-*Entamoeba histolytica* antibody titers of ≥ 128 by indirect hemagglutination assay, among persons seeking voluntary counseling and testing (VCT) for human immunodeficiency virus (HIV) infection. Between April 2006 and September 2009, 57 of 4,802 persons (1.2%) seeking VCT services were seropositive for *E. histolytica* infection. Compared with 228 seronegative controls, case subjects were older (odds ratio [OR] for per 1-year increase, 1.098; 95% confidence interval [CI], 1.036, 1.165), less likely to hold bachelor degree or higher (OR, 0.359; 95% CI, 0.152, 0.846), and were more likely to be men who have sex with men (MSM) (OR, 8.382; 95% CI, 2.050, 34.266) and have oral-anal sex (OR, 4.016; 95% CI, 1.711, 9.427) in multiple logistic regression analysis. The MSM, fecal-oral contamination, lower educational achievement, and older age were associated with increased risk for amebiasis among persons seeking VCT for HIV infection.

INTRODUCTION

In western countries, previous studies have shown that infection with *Entamoeba dispar* that is non-pathogenic is common in men who have sex with men (MSM), whereas infection with *Entamoeba histolytica* that is pathogenic and invasive is rare.^{1–7} In several developed countries in Asia, studies have shown that *E. histolytica* infection has become an emerging parasitic infection in MSM, especially those who are human immunodeficiency virus (HIV) infected.^{8–14} In Japan, 80% of the annually reported 500–600 cases occurred in MSM¹⁰; and the seroprevalence of MSM was estimated 13.4–20.4% compared with 1.0% in heterosexuals and 0.8% in prostitutes.¹³ In Taiwan, prevalence and incidence of amebiasis are also significantly higher in HIV-infected MSM than healthy controls and heterosexuals.^{8,9,14} An increasing trend of invasive amebiasis has been reported in HIV-infected patients seeking HIV care in Korea and Taiwan.^{11,15} Similarly, recent diagnosis of cases of invasive amebiasis among MSM or bisexual females with or without traveling to areas endemic for amebiasis raises concerns of spread of *E. histolytica* infection among persons at risk for HIV transmission in Australia and Canada.^{16–18}

In a cross-sectional study using stool antigen detection and polymerase chain reaction (PCR) from Mexico,¹⁹ where amebiasis is endemic, investigators found that HIV-infected patients appeared to have a higher rate, though not statistically significant, of *E. histolytica* infection than their sexual partners or close contacts. Notwithstanding, such data as prevalence and risk factors of amebiasis are scarce in persons at risk for HIV transmission in developed countries.²⁰ In this study, we aimed to characterize the risk factors associated with *E. histolytica* infection in persons who sought voluntary counseling and testing (VCT) for HIV infection at a university hospital that also provides routine counseling and testing for amebiasis.

MATERIALS AND METHODS

Setting. In April 2006, a program of free of charge, anonymous VCT for HIV infection for persons who considered themselves at risk for HIV infection and related sexually transmitted diseases (STD) was implemented in the university hospital that is also the largest designated hospital for outpatient and inpatient HIV care. An anonymous, self-administered questionnaire interview was performed to obtain demographics, and information of sexual practices, risk behaviors for HIV and STD, previous history of STD diagnosis, condom use, and use of recreational drugs. The study was approved by the Research Ethics Committee of the hospital and subjects gave written informed consent that was signed using a code consisting of birth year and the initial alphabet and the last four digits of identification card number.

Laboratory investigations. After completion of the questionnaire interview and integrated pre- and post-testing counseling, blood specimens were collected for serologic tests that included HIV infection, syphilis, and amebiasis. Anti-HIV antibody was tested using particle agglutination (SFD HIV 1/2 PA, Bio-Rad FUJIREBIO, Japan) and HIV infection was confirmed using Western blot (MP Diagnostics HIV BLOT 2.2. MP Biomedicals Asia Pacific Pte Ltd., Singapore). Assays for Venereal Disease Research Laboratory (VDRL) and *Treponema pallidum* hemagglutination antibody (TPHA) were simultaneously performed for diagnosis of syphilis. The indirect hemagglutination (IHA) assay was used to detect anti-*E. histolytica* antibodies (Celloagnostics, Boehringer Diagnostics GmbH, Marburg, Germany). The results were available within 1 day of blood sampling and the clients would be informed of the results by cell phone. For those who were tested positive for HIV infection and syphilis, outpatient visit for further examinations and treatment would be arranged.

The persons who were tested positive for IHA, regardless of titers were encouraged to submit stool specimens collected on three consecutive days for specific amebic antigen assays. Specific amoebic antigen assays (*ENTAMOEBA* TEST, TechLab, Branchburg, NJ) to detect *E. histolytica* of stool specimens were performed by following the manufacturer's instructions. Stool specimens tested positive for *E. histolytica* antigen were further confirmed as *E. histolytica* by PCR as

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previously described.⁹ After a pilot study to assess the cost-effectiveness of detection of *E. histolytica* in stool specimens in those persons seeking VCT services and HIV care,²¹ we have found that *E. histolytica* in persons who were seronegative for *E. histolytica* was rarely detectable; among 345 persons who underwent IHA serological assays and specific stool amebic antigen assays, 24 of 36 (66.7%) who were seropositive for *E. histolytica* had intestinal amebiasis by antigen assays, compared with 2 of 309 (0.2%) who were seronegative (odds ratio [OR], 307; 95% confidence interval [CI], 64.9, 1,451).²¹ Therefore, those who were seronegative for *E. histolytica* were not requested to submit stool specimens while counseling for amebiasis was provided.

Amebiasis is a reportable disease in Taiwan. Those persons diagnosed with amebiasis will be reported to Centers for Disease Control, Taiwan and treatment with metronidazole 500 mg thrice daily for 7–10 days followed by luminal agents (iodoquinol or paromomycin) to eradicate intestinal carriage. Three consecutive stool specimens were collected 1 week after completion of luminal agents and were subjected to specific amoebic antigen assays. To prevent further transmission of *E. histolytica*, we encourage the persons with amebiasis to contact their identifiable sexual partners and examinations and treatment of amebiasis will be provided free of charge as well.

Case and control. A case of amebiasis was defined as presence of an IHA titer of ≥ 128 . Four control subjects who were seronegative for *E. histolytica* infection were selected for each case and matched only for date when the case subject sought VCT: two controls before and two controls after the date. Because higher IHA titers may persist for a certain period of time, only the first episode of an elevated IHA titer was included; the subsequent episodes of those subjects who were seropositive for *E. histolytica* and underwent repeat VCT for HIV infection (1 subject) were excluded from analysis.

Statistical analysis. Variables were analyzed with the statistical software package (SSPS), version 12.0 (SPSS, Chicago, IL). We conducted a bivariate analysis using χ^2 analysis or Fisher's exact test for categorical variables and

the Wilcoxon rank sum test for non-categorical variables. All comparisons were two-tailed and a P value < 0.05 was considered significant. Multivariate analysis was performed by a forward-conditional binary logistic regression method that incorporated the variables with P value < 0.10 in the bivariate analysis.

RESULTS

From April 1, 2006 to September 30, 2009, 4,802 persons sought VCT services with 6,651 tests performed; 57 persons (1.19%) were seropositive for *E. histolytica* infection that was defined as having an IHA titer of ≥ 128 . Seroprevalence of *E. histolytica* infection was statistically significantly higher among MSM than among heterosexuals: 2.7% (9/338) versus 0.4% (3/750) in the first 14-month study period; 1.6% (21/1,316) versus 0.2% (2/1,218) in the second 14-month study period; 1.6% (21/1,930) versus 0.2% (2/1,099) in the third 13-month study period (Figure 1).

During the study period, 99 persons submitted at least one stool specimen for specific amoebic antigen assays for *E. histolytica*. Compared with the persons who were seronegative for *E. histolytica* or had lower IHA titers (< 128), persons who were seropositive had a significantly higher prevalence of intestinal infection with *E. histolytica*: 65.1% (28/43) versus 3.6% (2/56) ($P < 0.0001$); both cases of intestinal infection with *E. histolytica* occurring in persons who were seronegative for *E. histolytica* or had IHA titers < 128 had an IHA titer of 64. Intestinal infection with *E. histolytica* was associated with HIV infection among those 99 persons who submitted stool specimens: eight cases of HIV infection were detected among 32 persons (25.0%) who had intestinal infection with *E. histolytica* versus four cases of HIV infection among 67 persons (6.0%) who had no intestinal infection with *E. histolytica* ($P = 0.02$). All of the case subjects with *E. histolytica* identified in the stool specimens by antigen assays received metronidazole and paromomycin or iodoquinol and follow-up examinations indicated eradication of intestinal *E. histolytica* infection.

FIGURE 1. Seroprevalence of amebiasis defined as an indirect hemagglutination antibody titer of 128 or greater among persons receiving voluntary counseling and testing for human immunodeficiency virus (HIV) infection. MSM = men who have sex with men. This figure appears in color at www.ajtmh.org.

For the 57 case subjects, 228 control subjects were identified. The clinical and demographic characteristics of the 285 subjects are shown in Table 1. Compared with control subjects, case subjects were more likely to be older (34.5 versus 30.5 years, $P = 0.001$) and MSM (89.5% versus 50.9%, $P < 0.0001$), to have lower educational achievement (bachelor degree or higher, 54.4% versus 71.1%, $P = 0.03$), to report having oral-anal sex (50.9% versus 15.8%, $P < 0.0001$), oral-genital sex (84.2% versus 68.9%, $P = 0.02$), or receptive anal sex (71.9% versus 36.0%, $P < 0.0001$) within the past 3 months, and to be seropositive for syphilis (8.8% versus 2.6%, $P = 0.04$) and HIV infection (17.5% versus 3.5%, $P < 0.0001$). Consistent use of condom in oral-genital sexual contact (defined as use of condom in more than 75% of the sexual encounters within the past 3 months) was low in both groups and case subjects were significantly less likely to use condoms during oral sex than controls (1.8% versus 10.1%, $P = 0.01$).

In multiple logistic regression, we found that older age, MSM, oral-anal sexual contact, and lower educational achievement were four independent risk factors for amebiasis (Table 2). With per 1-year increase of age, the risk for amebiasis increased by nearly 10% (OR, 1.098; 95% CI, 1.036, 1.165). The MSM and subjects who practiced oral-anal sex had 8.382–(95% CI, 2.050, 34.266) and 4.016-fold (95% CI, 1.711, 9.427) greater risk for amebiasis than subjects who were heterosexuals and subjects without oral-anal sexual contact, respectively (Table 2). Although subjects with oral-genital sex, anal sex, HIV infection, and syphilis had an increased risk for amebiasis, they were not statistically significantly different between case and control subjects.

DISCUSSION

In this setting of VCT that may provide as an entry point for prevention and care for HIV infection and other sexually transmitted diseases, we found that MSM were at significantly higher risk for *E. histolytica* infection either by serologies or specific amebic antigen assays compared with those persons who were not MSM in Taiwan; in addition, intestinal infec-

TABLE 1
Comparisons of characteristics between cases of amebiasis (indirect hemagglutination antibody titers ≥ 128) and matched controls*

Variable	Case (N = 57)	Control (N = 228)	Statistics P =
Age, mean (range), yr	34.5 (21–55)	30.5 (19–65)	0.001
Male sex, N (%)	55 (96.5)	199 (87.3)	0.05
Men who have sex with men	51 (89.5)	116 (50.9)	< 0.0001
Married	6 (10.5)	34 (14.9)	0.06
Education achievement $\geq$ bachelor degree	31 (54.4)	162 (71.1)	0.01
Sexual partner > 1	51 (89.5)	207 (90.8)	0.8
Use of illicit drug	6 (10.5)	16 (7.0)	0.4
Previous history of STD	7 (12.3)	22 (9.6)	0.62
Oral-anal sex	29 (50.9)	36 (15.8)	< 0.0001
Oral-genital sex	48 (84.2)	157 (68.9)	0.02
Receptive or insertive anal sex	41 (71.9)	82 (36.0)	< 0.0001
VDRL ≥ 4	5 (8.8)	6 (2.6)	0.04
HIV infection	10 (17.5)	8 (3.5)	< 0.0001
Use of condom in oral sex on $\geq$ 75% of the occasions	1 (1.8)	23 (10.1)	0.01
Use of condom in anal sex on $\geq$ 75% of the occasions	22 (38.6)	42 (18.4)	0.5

*STD = sexually transmitted diseases; VDRL = Venereal Disease Research Laboratory; HIV = human immunodeficiency virus.

TABLE 2
Multiple logistic regression analysis of factors associated with amebiasis among persons seeking voluntary HIV counseling and testing*

Variable	Odds ratio	95% Confidence interval
Male gender	0.930	0.089, 9.692
Age (per 1-year increase)	1.098	1.036, 1.165
Education achievement $\geq$ bachelor degree	0.359	0.152, 0.846
No. of sexual partners > 1	0.990	0.947, 1.034
HIV infection	2.217	0.621, 7.912
MSM	8.382	2.050, 34.266
Oral-anal sex	4.016	1.711, 9.427
Oral-genital sex	1.493	0.470, 4.739
Anal sex	1.153	0.434, 3.064
VDRL ≥ 4	1.747	0.271, 11.254

*HIV = human immunodeficiency virus; MSM = men who have sex with men; VDRL = Venereal Disease Research Laboratory.

tion with *E. histolytica* was also associated with HIV infection when specific antigen assays were used to detect *E. histolytica*. These findings are of particular public health concerns and continued surveillance is indicated because male homosexual contact remains the leading route of HIV transmission and other STD in many developed countries and transmission of *E. histolytica* or other intestinal pathogens and HIV may be potentially facilitated in settings where unprotected oral-anogenital sex among MSM is likely to occur.²²

In this study, the overall prevalence of amebiasis by serologies is estimated 1.2% in persons seeking VCT services and 1.5–2.6% in MSM, which is lower than that in HIV-infected MSM seeking HIV care at our hospital (7.2%).⁹ The lower prevalence of amebiasis in those persons seeking VCT services than HIV-infected patients may be caused by referral bias in that most of the patients newly diagnosed with HIV infection referred to this hospital were at late stage of HIV infection.⁹ The other possible explanation may be that HIV-infected patients may have had increased frequency or intensity of risky exposure to both HIV and *E. histolytica* because both older age and HIV infection are associated with amebiasis in our study, though HIV infection itself did not reach statistical significance in multivariate analysis.

In this case-control study, our findings provide further support to the observation that MSM remain at significantly higher risk for amebiasis than heterosexuals in Taiwan, probably because of infrequent use of condoms during oral-anogenital sexual contact among MSM, which may increase risk for *E. histolytica* transmission.²² In this study, less than 5% of the case subjects who were predominantly MSM used condom consistently in oral-anogenital sexual contact. However, we were not able to show the statistical significance in this study, probably because of the small sample size or low specificity or sensitivity of the queries with respect to sexual practices and the time frame of the unprotected behaviors listed in the questionnaire. In this study, we did not specifically inquire and examine the interval between the latest risky sexual contact for HIV infection and amebiasis and seeking VCT services.

Oral-anal sexual contact with persons that are intestinal carriers of *E. dispar* appears to be the route of transmission in MSM who seek medical attention for STD in western countries.^{1,3,4,6,7} Because *E. dispar* and *E. histolytica* share the same transmission route, it is not unexpected that risk of *E. histolytica* infection will also increase in persons with risky sexual contact with individuals who reside in or travel to areas that

are endemic for *E. histolytica* infection.¹⁶ Most of the infections with *E. histolytica* are asymptomatic and carriage of *E. histolytica* may be prolonged,²³ and therefore, spread of *E. histolytica* is likely to occur in the gay community in endemic areas for *E. histolytica* infection when unprotected oral-anogenital sexual practices are adopted. In previous study, we have demonstrated that case cluster of *E. histolytica* infection might have occurred in HIV-infected patients in Taiwan.⁹ Similarly, case clustering may also occur in this high-risk population who sought VCT, although we did not further analyze the genetic relatedness of the isolates of *E. histolytica*. Because of the association between HIV infection and intestinal infection with *E. histolytica*, invasive amebiasis, which may be life-threatening, may be more likely to develop once HIV infection and progressive immunosuppression occur. Therefore, similar to other STD, screening and counseling for *E. histolytica* infection among MSM should be provided to prevent infection and development of invasive diseases because those carriers of *E. histolytica* may potentially serve as a reservoir to transmit *E. histolytica* to other MSM by oral-anogenital sexual contact.

There are several limitations in our study and interpretation of the results should be cautious. First, amebiasis was defined as presence of an IHA titer of ≥ 128 in this study. Antibodies can persist for years after infection and this fact limits their usefulness for diagnosing currently active disease in endemic countries. Second, although the specificity of IHA for *E. histolytica* is very high, the sensitivity of IHA is low compared with specific amoebic antigen assays, which could lead to an information bias that would affect both the selection of cases and the controls. Third, the association between seropositivity for *E. histolytica* infection and homosexuality is only reported in East Asia (Taiwan and Japan); more studies from other countries are needed to confirm our findings using both serologies and specific amoebic antigen assays. Fourth, limited by the design questionnaire, we are not able to explain why older age and lower education achievement were associated with amebiasis, although we postulate that increased cumulative exposure to *E. histolytica* with age and poorer adherence to hygiene among subjects with lower education achievement may be contributory.

In conclusion, male homosexuality, older age, and lower education achievement were associated with higher risk for amebiasis in persons who sought VCT services. It is important for health care providers to diagnose amebiasis and provide appropriate treatment and counseling to MSM in preventing development of invasive amebiasis and transmission of *E. histolytica*.

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