Seroprevalence of *Entamoeba histolytica* Infection among Chinese Men Who Have Sex with Men

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Abstract

**Background:** Men who have sex with men (MSM) were found to be one of the high-risk populations for *Entamoeba histolytica* (*E. histolytica*) infection. Accompanied by the prevalence of human immunodeficiency virus (HIV) among MSM, invasive amebiasis caused by *E. histolytica* has been paid attention to as an opportunistic parasitic infection. However, the status of *E. histolytica* infection among MSM has been barely studied in mainland China.

**Methods:** Seroprevalence of *E. histolytica* was determined using an enzyme-linked immunosorbent assay based on a cross-sectional study conducted in Beijing and Tianjin, China. Factors potentially associated with *E. histolytica* infection were identified by logistic regression analysis.

**Results:** A total of 602 MSM were included in the study and the laboratory data on serostatus of *E. histolytica* were available for 599 of them (99.5%). 246 (41.1%) and 51 (8.5%) of the study participants were *E. histolytica* seropositive and HIV seropositive, respectively. Univariate analyses suggested preferred anal sex behaviors were associated with *E. histolytica* seropositivity. In multivariate logistic regression analysis, “only has receptive anal sex” (OR: 2.03; 95% CI: 1.22 3.37), “majority receptive anal sex” (OR: 1.83; 95% CI: 1.13, 2.95), and “sadomasochistic behavior (SM)” (OR: 2.30; 95% CI: 1.04, 5.13) were found to be significantly associated with *E. histolytica* infection.

**Conclusions:** High seroprevalence of *E. histolytica* infection was observed among MSM from Beijing and Tianjin, China. Receptive anal sex behavior and SM were identified as potential predictors. Therefore, *E. histolytica* and HIV co-infection needs to be concerned among MSM due to their sharing the common risk behaviors.

Introduction

*Entamoeba histolytica* (*E. histolytica*) has a worldwide distribution and is endemic in most developing countries. Invasive amebiasis (IA) caused by *E. histolytica* is a very common human gastrointestinal parasitic disease which affected 50 million people worldwide and caused greater than 100,000 deaths annually. High risk populations for developing IA include infants, travelers from endemic area, and patients who are taking immunosuppressant. In mainland China, *E. histolytica* infection was also very prevalent in general population. The average prevalence of *E. histolytica* infection was 0.95%, ranged from 0.01% to 8.12% [3].

In 1967, the association between amebiasis and homosexuality was suggested for the first time [4]. Men who have sex with men (MSM) population had already been found to be a high risk population with *E. histolytica* infection before 1990. Homosexuality and oral-anal sex have been most frequently reported as potential risk factors for *E. histolytica* infection [5–13]. Accompanied by the transmission of human immunodeficiency virus (HIV) in MSM population, the prevalence of IA caused by *E. histolytica* are increasing and getting the attention as an important opportunistic parasitic infection. Recent studies from Australia, Japan, Korea and Taiwan reported increased risks for *E. histolytica* infection and IA among HIV-positive MSM [14–18]. Hung CC and colleagues recently reviewed the status of *E. Histolytica* infection in MSM [19]. By the end of 2011, China had about 780,000 people living with HIV/AIDS and 17.4% of them were MSM. The estimated new HIV infections in 2011 are 48,000 and 38.1% were MSM [20]. Case report of IA suggested the risk of *E. histolytica* prevalence among Chinese MSM, especially in the HIV positives [21]. However, to our knowledge, the prevalence of *E. histolytica* infection among MSM population has not been investigated in mainland China. The major aim of this study was to assess the seroprevalence of *E. histolytica* infection and the potential impact factors among MSM from China.

Methods

Ethics statement

The study was approved by the Ethics Committees of the Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College. All participants were the adults of men who...
had sex with men. Written informed consent was obtained before the interview and testing.

Study design and participants
A cross-sectional study was conducted in Beijing and Tianjin, China. Six hundred study participants were recruited between March and July 2010, though local non-government organizations (Beijing Rainbow Volunteers Workstation and Tianjin Deep Blue Volunteers Workgroup). Participants’ recruitment and inclusion criterion had been addressed in previous study [22].

Data collection
Questionnaire administered by the trained interviewer were performed for each study participant, the data acquired from the questionnaire includes socio-demographic characteristics (e.g., age, income, ethnicity, education, employed, and marital status), sexual orientation and homosexual act, sexual behaviors in the past 6 months, history of STDs and HIV infection. Anilingus behaviors were defined as sexual stimulation involving oral contact with the anus and sadomasochistic behaviors (SM) were defined as behaviors which aimed to enhance sexual gratification from inflicting or submitting to physical and emotional abuse.

Sample collection and laboratory tests
Blood samples were collected for *E. histolytica* and HIV serology. Serum samples were stored at −80°C until tested. Each study participant was assigned a unique identification number that was used to link the questionnaire and specimens. The HIV infection status was screened by an enzyme immunoassay (Wantai Biological Medicine Company, Beijing, China), and positive tests were confirmed by HIV-1/2 Western blot assay (HIVBlot 2.2 WB; Genelabs Diagnostics, Singapore). Qualitative screenings of serum immunoglobulin G (IgG) antibodies to *E. histolytica* were retrospectively performed using the commercial enzyme-linked

| Factors                  | Beijing (N = 302) | Tianjin (N = 300) | p for difference |
|--------------------------|------------------|-------------------|-----------------|
| **Age**                  |                  |                   |                 |
| ≤19 years                | 17 (6.0)         | 18 (6.3)          |                 |
| 20–29 years              | 184 (60.9)       | 199 (66.3)        |                 |
| 30–39 years              | 66 (21.9)        | 45 (15.0)         |                 |
| ≥40 years                | 34 (11.3)        | 37 (12.3)         | 0.194           |
| **Ethnicity**            |                  |                   |                 |
| Han                      | 285 (94.7)       | 285 (95.3)        |                 |
| Others                   | 16 (5.3)         | 14 (4.7)          | 0.722           |
| **Education**            |                  |                   |                 |
| ≤9 years                 | 64 (21.3)        | 44 (14.7)         |                 |
| 10–12 years              | 93 (31.0)        | 90 (30.0)         |                 |
| >12 years                | 143 (47.7)       | 166 (55.3)        | 0.065           |
| **Marriage status**      |                  |                   |                 |
| Unmarried                | 232 (77.1)       | 235 (78.3)        |                 |
| Married/divorced/widowed | 69 (22.9)        | 65 (21.7)         | 0.711           |
| **HIV-1 serostatus**     |                  |                   |                 |
| Negative                 | 268 (89.9)       | 279 (93.0)        |                 |
| Positive                 | 30 (10.1)        | 21 (7.0)          | 0.179           |
| **Entamoeba histolytica serostatus** |        |                   |                 |
| Negative                 | 181 (60.1)       | 172 (57.7)        |                 |
| Positive                 | 120 (39.9)       | 126 (42.3)        | 0.548           |

Abbreviation: HIV, human immunodeficiency virus.
*Sum may not always add up to total because of missing data.

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immunosorbent assay (ELISA) kit (Shanghai Fengxiang Biological Technology Co. Ltd. Shanghai, China). Purified *E. histolytica* antigen was used to coat microtitration wells, incubated for 30 minutes at 37°C after adding 10 μL serum samples to wells. Washing and removing non-combinative antibody and other components, then combined HRP-conjugate reagent, then incubated and washed again. The substrate solution was added to each well, after 15 minutes at 37°C, stop solution was added to arrest color development and a ELISA reader was used to measure the absorbance at 450 nm. Each sample was tested in duplicate and the average optical density (OD) value was calculated. Test validity was evaluated as: the average of the positive controls should $\geq 1.00$ and the average of negative controls should $\leq 0.10$. The cut off value was set as the average of negative controls $+0.15$ according to the instruction of the kit.

**Statistical analysis**

Questionnaires were double entered and compared with EpiData software (EpiData 3.02 for Windows, The Epi Data Association Odense, Denmark). After cleaning, the data were then converted and analyzed using Statistical Analysis System (SAS 9.2 for Windows; SAS Institute Inc., NC, USA).

Study population was characterized by site with respect to age, ethnicity, education, marriage status, and current status of HIV. Differences between sites in these variables were assessed with Pearson chi-square test. The associations of *E. histolytica* infection with the characteristics of demographics, sexual behaviors, diagnosed STDs including HIV, and current status of HIV were estimated using Pearson chi-square test. Variables related with *E. histolytica* serology ($p<0.1$) in the univariate analyses were included in a multiple logistic regression model additionally adjusted for age, site, and HIV infection status. The Cochran-Armitage test was used to evaluate the trend of OD value in *E. histolytica* antibody test associated with anal sex behaviors categories.

**Results**

**Demographic characteristics and its association with *E. histolytica* infection**

A total of 607 participants were interviewed and signed the informed consent, 6 of them were excluded (3 did not complete sample collection and 2 Vietnamese living in Beijing). Finally, 602 were used for the analyses (302 from Beijing and 300 from Tianjin). Laboratory data on serostatus of *E. histolytica* and HIV were available for 599 (99.5%) and 598 (99.3%) of the study participants respectively. Age, ethnicity, education, marriage status, HIV-1 serostatus and *E. histolytica* serostatus of the study population were compared by site (Table 1). No significant difference was found for any characters ($p>0.05$). Therefore, participants from the two sites were pooled together for further association analyses. Age of the participants ranged from 16 to 72 years, with a mean age of 27.9±8.3 years. The participants showed the following characteristics: 95% (570/600) were Han nationality; 51.5% (309/600) had more than 12 year’s education; Table 2. Associations between demographical characteristics and *E. histolytica* serostatus.

| Factors                      | *E. histolytica* Seropositivity n/N (%) | p for difference |
|------------------------------|----------------------------------------|------------------|
| Age                          |                                        |                  |
| ≤19 years                    | 13/35 (37.1)                           |                  |
| 20–29 years                  | 166/381 (43.6)                         |                  |
| 30–39 years                  | 42/111 (37.8)                          |                  |
| ≥40 years                    | 25/70 (35.7)                           | 0.474            |
| Han Ethnic                   |                                        |                  |
| Not                          | 14/29 (48.3)                           |                  |
| Yes                          | 232/568 (40.9)                         | 0.428            |
| Site                         |                                        |                  |
| Beijing                      | 120/301 (39.9)                         |                  |
| Tianjin                      | 126/298 (42.3)                         | 0.548            |
| Registered residence         |                                        |                  |
| Not                          | 93/245 (38.0)                          |                  |
| Yes                          | 153/353 (43.3)                         | 0.188            |
| Education                    |                                        |                  |
| ≤9 years                     | 63/45 (41.7)                           | 0.388            |
| 10–12 years                  | 68/183 (37.2)                          |                  |
| >12 years                    | 133/306 (43.5)                         |                  |
| Marital status               |                                        |                  |
| Single                       | 192/464 (41.4)                         |                  |
| Married/divorced/widowed     | 54/134 (40.3)                          | 0.823            |
| Alcohol use                  |                                        |                  |
| No                           | 166/404 (41.1)                         |                  |
| Yes                          | 80/194 (41.2)                          | 0.973            |

*Sum may not always add up to total because of missing data.
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### Table 3. Associations between sexual behaviors and *E. histolytica* serostatus.

| Factors                                           | *E. histolytica* Seropositivity n/N* (%) | p for difference |
|---------------------------------------------------|----------------------------------------|------------------|
| **Self-reported sexual orientation**              |                                        |                  |
| Homosexual                                        | 181/424 (42.7)                         |                  |
| Bisexual or uncertain                              | 65/173 (37.6)                          | 0.249            |
| **Age at the first homosexual act**               |                                        |                  |
| <18 years                                         | 31/71 (43.7)                           |                  |
| ≥18 years                                         | 215/526 (40.9)                         | 0.654            |
| **Seeking sexual partners in gay venues**         |                                        |                  |
| No                                                | 120/265 (45.3)                         |                  |
| Yes                                               | 126/333 (37.8)                         | 0.066            |
| **Total number of MSM sex partners**              |                                        |                  |
| ≤10                                               | 123/297 (41.4)                         |                  |
| >10                                               | 123/301 (40.9)                         | 0.891            |
| **Frequency of sexual behavior in the past 6 months** |                                        |                  |
| ≤1 per month                                      | 61/133 (45.9)                          |                  |
| >1 per month                                      | 185/463 (40.0)                         | 0.223            |
| **Preferred anal sex behavior**                   |                                        |                  |
| Insertive only                                    | 45/147 (30.6)                          |                  |
| majority insertive                                | 46/120 (38.3)                          |                  |
| majority receptive                                | 77/169 (45.6)                          |                  |
| Receptive only                                    | 71/147 (48.3)                          | 0.009            |
| **Is oral sex a common sexual behavior?**         |                                        |                  |
| Not                                               | 71/166 (42.8)                          |                  |
| Yes                                               | 175/432 (40.5)                         | 0.615            |
| **Is anilingus a common sexual behavior?**        |                                        |                  |
| Not                                               | 168/415 (40.5)                         |                  |
| Yes                                               | 78/183 (42.6)                          | 0.624            |
| **SM**                                            |                                        |                  |
| No                                                | 229/568 (40.3)                         |                  |
| Yes                                               | 17/30 (56.7)                           | 0.076            |
| **Anal sex in the past 6 months**                 |                                        |                  |
| No                                                | 22/41 (53.7)                           |                  |
| Yes                                               | 224/555 (40.4)                         | 0.095            |
| **Consistent condom use in all style of homosexual behaviors in the past 6 months** | | |
| No                                                | 217/527 (41.2)                         |                  |
| Yes                                               | 3/7 (42.9)                             | 0.929            |
| **Group sex**                                     |                                        |                  |
| No                                                | 215/534 (40.3)                         |                  |
| Yes                                               | 29/61 (47.5)                           | 0.274            |
| **Having received money for sex with males**      |                                        |                  |
| No                                                | 227/552 (41.1)                         |                  |
| Yes                                               | 14/37 (37.8)                           | 0.694            |
| **Having paid sex with males**                    |                                        |                  |
| No                                                | 233/567 (41.1)                         |                  |
| Yes                                               | 8/22 (36.4)                            | 0.658            |
| **Ever had STDs including HIV-1**                 |                                        |                  |
| No                                                | 184/454 (40.5)                         |                  |
| Yes                                               | 56/133 (42.1)                          | 0.745            |
| **HIV-1 infection**                               |                                        |                  |
| No                                                | 221/545 (40.6)                         |                  |
77.7% (467/601) were single. Laboratory data suggested two hundred and forty six (24.3%) of participants with E. histolytica infection were homosex-uals. Age of the first homosexual act ranged from 5 to 55 years old, with a mean age of 21.4±5.0 years. The median number of their homosexual partners was eleven and 43.1% of participants had stable homosexual partners before baseline survey. In the past one year, sixty one participants (10.2%) had group sex, thirty eight participants (6.3%) had ever received money for sex with male partners, and twenty three (3.8%) had ever provided money for sex with male partners. In the past 6 months, 133 (22.2%) participants reported had sexual behavior less than 1 time per month and only 1.3% (8/602) of participants insisted on using condoms in the process of insertive or receptive anal sex and oral sex. 22.5% of participants had ever been diagnosed sexually transmitted diseases. The association between sex behaviors and E. histolytica infection were also assessed. Self-reported preferred anal sex behaviors were classified to four types (only has insertive anal sex, majority insertive anal sex, majority receptive anal sex, and only has receptive anal sex). Univariate analyses suggested preferred anal sex behaviors were associated with E. histolytica infection. HIV infection was not found associated with E. histolytica infection (Table 3).

Sexual behaviors and its association with E. histolytica infection

Homosexual men accounted for 71.0% (427/602), and bisexual men for 24.3% (146/602) of the study population. 73.6% (181/246) of participants with E. histolytica seropositive were homosex-

### Table 3. Cont.

| Factors                                      | E. histolytica Seropositivity n/N (%) | p for difference |
|----------------------------------------------|--------------------------------------|-----------------|
| **Heterosexual behaviors in the past 6 months** |                                      |                 |
| No                                           | 164/375 (43.7)                       |                 |
| Yes                                          | 82/223 (36.8)                        | 0.094           |
| **Had ever been circumcised**                 |                                      |                 |
| No                                           | 206/503 (41.0)                       |                 |
| Yes                                          | 28/57 (49.1)                         | 0.236           |

Abbreviation: SM, sadomasochism; STDs, sexually transmitted diseases.

*Sum may not always add up to total because of missing data.

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### Table 4. The associations of E. histolytica infection with potential factors in multivariate logistic regression model.

| Factors                                      | Ajusted OR (95% CI) * | p value |
|----------------------------------------------|-----------------------|---------|
| **Preferred anal sex behavior**              |                       |         |
| Insertive only                               | Ref.                  |         |
| Majority insertive                           | 1.43 (0.85, 2.40)     | 0.174   |
| Majority receptive                           | 1.83 (1.13, 2.95)     | 0.014   |
| Receptive only                               | 2.03 (1.22, 3.37)     | 0.006   |
| **Seeking sexual partners in gay venues**   |                       |         |
| No                                           | Ref.                  |         |
| Yes                                          | 0.74 (0.50, 1.08)     | 0.115   |
| **Anal sex in the past 6 months**            |                       |         |
| No                                           | Ref.                  |         |
| Yes                                          | 0.52 (0.25, 1.07)     | 0.075   |
| **SM**                                       |                       |         |
| No                                           | Ref.                  |         |
| Yes                                          | 2.30 (1.04, 5.13)     | 0.041   |
| **Heterosexual behaviors in the past 6 months** |                       |         |
| No                                           | Ref.                  |         |
| Yes                                          | 0.93 (0.63, 1.38)     | 0.713   |

Abbreviation: CI, confidential interval; OR, odds ratio; SM, sadomasochism. Anal sex in the past 6 months, preferred anal sex behavior, SM, seeking sexual partners in gay venues and heterosexual behaviors in the past 6 months were included in a multivariate logistic regression model, adjusted by age, site, and HIV infection status.

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significant predictors for *E. histolytica* infection. In addition, significant different antibody levels were observed between subgroups with respect to the preferred anal sex behavior.

The first observation of a relation between enteric protozoan infections and sexual behavior was reported in 1968 [4]. Epidemiological studies conducted in the developed countries showed homosexuals or MSM had significant higher risk of *E. histolytica* infection. Using microscopy for diagnosis, the prevalence varied from 20% to 32% among MSM without gastrointestinal symptoms [5,7,8,23–27]. However, microscopy is not sensitive or specific enough for the detection of *E. histolytica* in clinical specimens, especially for the differentiation *E. histolytica* from *E. dispar* and *E. moshkovskii* in the epidemiological studies with a large sample size. Therefore, serological tests were used to detect the infection though measuring anti-*E. histolytica* antibodies and seroprevalence ranging from 0.2% to 21% using ELISA test among HIV negative MSM were reported in several developed countries [18,19]. Our results, for the first time, suggested a high prevalence (41.1%) of *E. histolytica* infection among MSM community from China. A recently published study conducted among general population in seven provinces in China showed that the seroprevalence of *E. histolytica* infection varied from 6% to 11% [28]. Although antibody test could not distinguish the past or current infection status and maybe overestimated the epidemic status, the fact that amebic liver abscess and latent infection had become one of the common opportunistic infection diseases among Chinese MSM AIDS patients reminds us to pay attention to the co-infection of *E. histolytica* and HIV [21,29].

In the present study, homosexual behaviors were mostly classified according to participants’ tendency. Receptive anal sex behavior was found to be related to higher prevalence of *E. histolytica* infection. This finding and its underlying mechanisms should be further studied in the future. Homosexuals and history of anilingus had been demonstrated to be the risk factors of *E. histolytica* infection. In 1978, a study from the New York city reported that 20% of eighty nine sexually active homosexual men had amebiasis and the presence of infections associated with history of anilingus [5]. Another study from a venereal-disease-clinic population compared the prevalence of *E. histolytica* infections in homosexual men, bisexual men and heterosexual men. Homosexuality and oral-anal sex were found to be the most important risk factors for *E. histolytica* infection [7]. However, such an association was not observed in our study population.

Interestingly, SM was found to be associated with *E. histolytica* infection in the present study. The data on the specific behaviors during the process of SM has not been well studied in China due to the potential issues of social culture and discrimination. Several published studies had revealed that people who had engaged in SM were more likely to have experienced oral-anal sex and other sexual risk practice [30–32]. In addition, fecal-oral contamination in these sexual behaviors maybe occurs and increases the opportunity of pathogen infection. Keystone JS’ study showed
cleaning of anus before anal sex was associated with a significant lower prevalence of infection [23]. But it is difficult to explore the factors linked SM behaviors to the infection susceptibility in our present study because only 30 participants (5.0%) reported SM behaviors. Developing targeted prevention and control strategies, such as promoting sanitary habit before sexual behavior, may decrease the opportunity of pathogen infection. Therefore, it is necessary to further study potential risky behaviors associated with health problems among Chinese MSM.

Several limitations of this study should be kept in mind. First, potential bias due to the inaccurate response to the questionnaire, especially to the questions on sexual behaviors, could not be excluded completely. Second, our study participants might not represent the general MSM population due to the potential limitation of enrollment methods. Therefore, potential selection bias should be considered when interpret our results. Third, serology could not clearly identify the infection status as current infection or past infection, potential bias caused by such misclassification could not be excluded. Although statistically significant difference of antibodies levels was observed with respect to the preferred anal sex behaviors (p<0.001), however, the smaller sample size in each subgroups and a broad range of OD value should be considered. Further studies are needed to explore the underlying mechanisms for the observed relation between receptive anal sex behaviors and *E. histolytica* infection. Fourth, cross-sectional study design has its limitation on association analysis. Therefore, our results need confirmation by further large-scale case-control studies or prospective studies.

In conclusion, high prevalence of *E. histolytica* infection was observed among MSM from Beijing and Tianjin, China. Receptive anal sex behaviors and SM were identified as significant predictors for *E. histolytica* infection. Prevention and control of *E. histolytica* infection among Chinese MSM should be concerned because this special population confronted with high risk of HIV infection.

Supporting Information

Checklist S1  STROBE Checklist. (DOC)

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Author Contributions

Conceived and designed the experiments: LG QJ. Performed the experiments: ML XL YY. Analyzed the data: FZ. Contributed reagents/materials/analysis tools: ML CG. Wrote the paper: FZ LG.