Cost-Effectiveness of Detection of Intestinal Amebiasis by Using Serology and Specific-Amebic-Antigen Assays among Persons with or without Human Immunodeficiency Virus Infection[▽]

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Among 345 persons who underwent indirect hemagglutination (IHA) serological assays and assays of specific amebic antigens in their stool samples, 24 of 36 (66.7%) who were seropositive for *Entamoeba histolytica* had intestinal amebiasis as determined by antigen assays compared with 2 of 309 (0.2%) who were seronegative (odds ratio, 307; 95% confidence interval, 64.9 to 1,451). The estimated cost to detect a case of intestinal amebiasis by serology followed by antigen assays (\$52) could be reduced by 74.3% and 69.9%, respectively, compared with the costs of the concurrent use of both assays (\$202) and the antigen assays alone (\$173). Our finding suggests that IHA assays followed by specific-amebic-antigen assays can be cost-effective in the diagnosis of intestinal amebiasis among persons with or without human immunodeficiency virus infection who are at risk for *E. histolytica* infection.

In East Asia, *Entamoeba histolytica* infection (amebiasis) has become an emerging parasitic infection among men who have sex with men (MSM), especially those who are human immunodeficiency virus (HIV) infected (4–6, 9–14, 16). In Japan, 80% of the annually reported cases of amebiasis occurred in MSM (8), and the seroprevalence of amebiasis in MSM was estimated to be 13.4% to 20.4%, compared with 1.0% in heterosexuals and 0.8% in prostitutes (12–14). In Taiwan, the prevalence and incidence of amebiasis are also significantly higher among HIV-infected MSM than among healthy controls and heterosexuals (5, 6, 16). Therefore, it is important for health care providers to diagnose amebiasis and provide appropriate treatment and counseling to MSM to prevent the development of invasive amebiasis and the transmission of *E. histolytica*.

The tests used to diagnose amebiasis include microscopy of clinical samples, serology, specific-amebic-antigen assays, and molecular methods of testing stool and liver aspirate samples (3, 15). Of those tests, specific-amebic-antigen assays of stool samples have the highest sensitivity and specificity in the diagnosis of intestinal amebiasis (2, 15); however, their cost is significantly higher than microscopy and serology (2, 15). How to maximize the cost-effectiveness of serological tests and amebic-antigen assays for the diagnosis of intestinal amebiasis has not been investigated before. In this study, we aimed to compare the costs and diagnostic yields of serological tests combined with amebic-antigen assays among persons at risk for HIV infection and amebiasis.

(Preliminary analyses of these data were presented at the 18th

European Congress of Clinical Microbiology and Infectious Diseases held in Barcelona, Spain, on 19 to 22 April 2008 [1].)

Between January 2006 and February 2008, 376 HIV-infected persons who sought HIV care and 2,511 non-HIV-infected persons who sought anonymous HIV testing at the National Taiwan University Hospital were enrolled in the study. At least one stool sample and one blood sample from each person were obtained for specific-amebic-antigen assays and indirect hemagglutination (IHA) assays, respectively (Fig. 1). IHA assays to detect anti-*E. histolytica* antibodies were performed by following the instructions of the manufacturer (Celloagnostics; Boehringer Diagnostics GmbH, Marburg, Germany). Seropositivity was defined as a titer of 128 or greater. The purchase cost for each test was estimated to be \$1.7; three additional tests (\$5.1) are needed to confirm an IHA titer of 128 or greater. To detect *E. histolytica* in stool samples, specific-antigen assays were performed using commercial kits (*Entamoeba* test; TechLab, Branchburg, NJ). The purchase cost for each antigen test was estimated to be \$13. To confirm the presence of *E. histolytica*, PCRs were performed using primers specific for *E. histolytica* as described previously (6). The primer sets for a multiplex nested PCR were based upon the variable regions between 16S-like ribosomal DNAs of *E. histolytica* (GenBank accession no. X56991) and *E. dispar* (GenBank accession no. Z49256).

The characteristics of the study populations are shown in Table 1 and Fig. 1. The seroprevalence of amebiasis was 3.8% (47/1,237) (95% confidence interval [CI], 2.9 to 5.0) among MSM compared with 0.36% (6/1,650) (95% CI, 0.15 to 0.75) among individuals not in the MSM group (non-MSM individuals) and those with unknown risk factors ($P < 0.001$). HIV-infected MSM had the highest seroprevalence of *E. histolytica* infection (10.1%; 28/277) compared with non-HIV-infected

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FIG. 1. Results for the study population of men with amebiasis, whose sera were subjected to IHA assays and whose stool samples were subjected to specific-amebic-antigen assays.

MSM (2.1%; 19/960) and non-HIV-infected non-MSM individuals (0.2%; 3/1,551).

A total of 426 persons submitted stool samples for amebic-antigen assays. Their clinical characteristics are shown in Table 1. The prevalence of intestinal amebiasis was 9.3% (27/291) (95% CI, 6.4 to 13.2) among MSM compared with 2.2% (3/135) (95% CI, 0.5 to 6.6) among non-MSM individuals and

those with unknown risk factors ($P = 0.004$). The prevalence of intestinal amebiasis was 25.7% (9/35) among non-HIV-infected MSM, 7.0% (18/256) among HIV-infected MSM, and 0% (0/98) among non-MSM individuals or those with unknown risk factors. The higher prevalence of intestinal amebiasis among non-HIV-infected MSM than among HIV-infected MSM might be related to the fact that those non-HIV-infected MSM were more willing to submit stool samples for amebic-antigen assays once they learned that they were seropositive for *E. histolytica*.

During the same study period, specimens from a total of 345 persons were subjected to both IHA and amebic-antigen assays (Table 1 and Fig. 1); 36 of them (10.4%; 95% CI, 7.5 to 14.0) had high IHA titers. Seropositivity for *E. histolytica* was statistically significantly associated with the presence of *E. histolytica* in the stool samples. Among the 36 persons with high IHA titers, 24 (66.7%) also tested positive for *E. histolytica* by antigen assays compared with 2 of 309 (0.6%) who had low or negative IHA titers (odds ratio, 307; 95% CI, 64.9 to 1,451) (Fig. 1). Of the 26 persons who tested positive for *E. histolytica* by antigen assays (including 2 persons who were seronegative for *E. histolytica*), 21 submitted a second stool sample for PCR. Of the 21 antigen-positive stool samples, the presence of *E. histolytica* was confirmed by PCR in 17 (81.0%), and all 17 samples were from persons seropositive for *E. histolytica*.

Based on the finding described above, we estimated that the cost to detect a case of intestinal amebiasis by IHA assays [$(\$1.70 \times 345 \text{ tests}) + (\$1.70 \times 3 \times 36 \text{ tests}) = \770] followed by amebic-antigen assays of the stool samples for those who were seropositive for *E. histolytica* ($\$13 \times 36 \text{ tests} = \468) was $\$52 [(\$770 + \$468)/24]$. By concurrent use of serology and

TABLE 1. Characteristics of the subjects who submitted stool and blood samples for specific-amebic-antigen assays and IHA assays

Variable	Value for persons submitting ^a :		
	Blood specimens for IHA assays	Stool specimens for antigen assays	Both specimens for antigen and IHA assays
Total no. of subjects	2,887	426	345
Median age (range) (yr)	28 (15–83)	37 (15–93)	36 (15–83)
Males	2,453 (85)	405 (95.1)	334 (96.8)
MSM	1,237 (42.8)	291 (68.3)	249 (72.2)
Non-MSM individuals	1,632 (56.5)	98 (23.0)	96 (27.8)
Subjects of unknown sexual behavior	18 (0.6)	37 (8.7)	0
HIV infected	376 (13.0)	358 (84.0)	299 (86.7)
MSM	277 (73.7)	256 (71.5)	214 (71.6)
Non-MSM individuals or unknown	99 (26.3)	102 (28.5)	85 (28.4)
Non-HIV infected	2,511 (87.0)	68 (16.0)	46 (13.3)
MSM	960 (38.2)	35 (51.5)	35 (76.1)
Non-MSM individuals or unknown	1,551 (61.8)	33 (48.5)	11 (23.9)
Antigen positive	NA	30/426 (7.0)	26/345 (7.5)
MSM	NA	27/291 (9.3)	25/249 (10.0)
Non-MSM individuals or unknown	NA	3/135 (2.2)	1/96 (1.0)
IHA titer of ≥ 128	53/2,887 (1.8)	NA	36/345 (10.4)
MSM	47/1,237 (3.8)	NA	34/249 (13.7)
HIV infected	28/277 (10.1)	NA	24/214 (11.2)
Non-HIV infected	19/960 (2.0)	NA	10/35 (28.6)
Non-MSM individuals or unknown	6/1,650 (0.4)	NA	0/96 (0)

^a All values are numbers (percentages) of patients except where indicated otherwise. NA, not applicable.

amebic-antigen assays and by amebic-antigen assays alone, the cost was estimated to be \$202 $[(\$770 + 13 \times 345)/26]$ and \$173 $[(13 \times 345)/26]$, respectively. Therefore, the cost to detect a case of intestinal amebiasis by serological tests followed by specific-amebic-antigen assays could be reduced by 74.3% and 69.9% compared with the costs of concurrent use of serology and amebic-antigen assays and the use of amebic antigen assays alone, respectively.

In this study, we found that MSM were at a higher risk for *E. histolytica* infection either by serology or amebic-antigen assays than persons who were not MSM in Taiwan, probably because of infrequent use of condoms during oral-anogenital sexual contact among MSM (7), which may increase the risk for *E. histolytica* transmission. Therefore, screening and counseling for *E. histolytica* infection among MSM should be provided to prevent infection and the development of invasive diseases, because those carriers of *E. histolytica* may potentially serve as a reservoir for the transmission of *E. histolytica* to other MSM via oral-anal sex.

Screening using stool samples can be cumbersome compared with screening using blood samples because stool samples may not be as readily available as blood samples in the clinical-care setting. Our finding that seropositivity for *E. histolytica* is highly associated with intestinal *E. histolytica* infection among subjects consisting of HIV-infected MSM, non-HIV-infected MSM, and non-HIV-infected non-MSM individuals suggests that the serological test may be used as a complementary tool and may provide a more cost-effective way to understand the epidemiology of intestinal *E. histolytica* infection among high-risk populations (6).

In this study of a population with a high seroprevalence of *E. histolytica* infection (10.1%), we estimated that to identify one case of intestinal amebiasis, the cost can be reduced by 69.9% to 74.3% if the algorithm is to test with serological assays first and then with amebic-antigen assays for those who are seropositive instead of concurrently using serology and amebic-antigen assays or using antigen assays only. Based on two assumptions, namely, that among those persons who are seropositive for *E. histolytica*, two-thirds will test positive for *E. histolytica* by antigen assays and that the prevalence of intestinal *E. histolytica* infection as determined by antigen assays in persons who are seronegative is approximately 0.6%, as demonstrated in this study, we estimate that the cost can be reduced by approximately 30% by using sequential assays rather than a combination of serological and antigen assays or antigen assays alone in a population with a low seroprevalence for *E. histolytica* infection (0.2% in non-HIV-infected MSM) and by 80% in a population with an intermediate seroprevalence (2.1% in non-HIV-infected MSM) (data not shown).

There are several limitations of our study, and interpretations of the results should be cautious. First, the prevalence of *E. histolytica* infection is higher among our subjects, who are mainly HIV-infected MSM living in an area that used to have a higher prevalence of amebiasis, than among the general population (16). The results of the cost-effectiveness analysis may not be generalizable to a population consisting mainly of non-HIV-infected persons or to other areas showing a different epidemiology of *E. histolytica* and HIV infection. Second, our subjects had asymptomatic intestinal colonization with *E.*

histolytica, and the results may not be generalizable to subjects with amebic colitis or liver abscess. Third, we did not include microscopy as a diagnostic tool for intestinal amebiasis. However, microscopy still has its role in the detection of other intestinal infections that are transmitted via the fecal-oral route (2). Therefore, our finding can be considered generalizable only in the context of intestinal amebiasis among MSM. Fourth, in the estimation of cost, we did not take into consideration costs related to equipment, personnel, other administrative issues, or PCR. Last, the shedding of *E. histolytica* may be intermittent, which may reduce the sensitivity of antigen assays if only one stool sample is tested.

In conclusion, our finding suggests that IHA assays followed by specific-amebic-antigen assays can be cost-effective in the diagnosis of intestinal amebiasis among persons at risk for HIV infection in an area of a high prevalence of *E. histolytica* infection among MSM.

We declare that no competing interests exist.

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