Seroprevalence of *Entamoeba histolytica* Infection in China

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Abstract. The seroprevalence of *Entamoeba histolytica* infection in the residents of seven provinces in China was examined by using an enzyme-linked immunosorbent assay with a crude antigen and a recombinant surface antigen, C-Ig1, of the parasites. A total of 1,312 serum samples were investigated. The positivity rates for these two antigens were 11.05% and 6.25%, respectively. There was no significant difference in the seropositivity to *E. histolytica* between men and women. We used a logistic regression model and maximal-likelihood methods to estimate the prevalence of *E. histolytica* infection from sequential serologic data. Seropositivity in Sichuan, Guizhou, and Sinkiang Provinces was higher than that in Beijing, Shanghai, and Qinghai Provinces. The present study provides an overview of seropositivity to *E. histolytica* infection in seven provinces in China and use the logistic regression model estimation method to achieve a more accurate measure of amebiasis prevalence.

INTRODUCTION

Amebiasis is an infection caused by the protozoan parasite *Entamoeba histolytica*.¹ Amebiasis is a major human gastrointestinal infection affecting approximately 50 million persons worldwide and accounts for more than 100,000 deaths annually.^{2,3} Another intestinal ameba, *E. dispar*, is morphologically indistinguishable from *E. histolytica*, but is nonpathogenic.⁴ *Entamoeba histolytica* is unique among amebae because of its ability to disrupt the intestinal mucosa, causing intestinal disease, amebic colitis, and the capacity for further hematogenous spread, thereby producing potentially fatal abscesses and extraintestinal diseases.^{2,4,5}

Amebiasis is a common problem in developing countries.⁶ The infection is initiated when mature cysts are ingested by hosts.⁷ Amebiasis is extremely transmissible, with an infectious dose as low as one viable cyst, and is usually acquired from food or water sources contaminated with feces.⁸ The prevalence rate of this disease is up to 6% in areas that have poor sanitation.⁹ For example, in a rural community in Mexico, 13.8% of the total population was shown to have asymptomatic *E. histolytica* infection by a polymerase chain reaction. Furthermore, a high rate of asymptomatic infection (21.4%) was detected fecal samples of persons from Africa and Egypt by an enzyme-linked immunosorbent assay (ELISA).^{10,11} The infection patterns differ between developing and developed countries. In the United States and Australia, persons at risk for *E. histolytica* infection include immigrants, travelers returning from countries with high endemicity, indigenous persons, and men who have sex with men.^{12,13}

However, no data on *E. histolytica* infection in China are available, except for information on fecal examinations obtained during the first national survey of human parasites in 1992.¹⁴ Therefore, the current preliminary study was conducted to estimate the seroprevalence of *E. histolytica* infection in a population in China. The known symptoms of *E. histolytica* infection include diarrhea, abdominal pain, and fever. Estimates show that in 90% of human cases, the infection is self-limiting and asymptomatic.¹⁵ However, positive serologic

results are found in most asymptomatic cases infected with *E. histolytica*; thus, serologic tests are also applicable.¹⁶ Serodiagnosis is an important laboratory diagnostic tool for amebiasis, as well as for microscopic detection of pathogens.

Recently, several recombinant *E. histolytica* antigens have been prepared, and their usefulness for serodiagnosis has been reported.^{17–19} One of these useful antigens is the glycosylphosphatidylinositol-anchored 150-kD intermediate subunit (Igl) of galactose- and N-acetyl-D-galactosamine-inhibitable lectin. Recombinant Igl is well recognized in serum samples from patients with amebiasis, but not from patients with other protozoan infections. The C-terminus fragment of Igl is valuable for serodiagnosis of amebiasis.¹⁹ In the present study, antibodies against *E. histolytica* were examined by ELISA using crude *E. histolytica* antigen and the recombinant fragment of the C-terminus fragment of Igl (C-Igl) of *E. histolytica*. A total of 1,312 serum samples collected from seven provinces in China had been examined.

MATERIALS AND METHODS

Ethical considerations. The serum samples were provided by the National Institute of Parasitic Diseases, Chinese Center of Disease Control and Prevention (CCDC). The CCDC obtained and stored the serum samples from donors during the survey organized and conducted by the Chinese Ministry of Health during 2001–2004. Simple random sampling was conducted to select participants. The Medical Ethics Committee of Fudan University approved the use of the serum samples in the current study, and written informed consent was obtained from the donors. All samples were from rural or urban–rural-integration areas.

Materials. The serum samples were provided by the National Institute of Parasitic Diseases, CCDC. Crude antigen was prepared from the trophozoites of *E. histolytica* strain HK9 axenically cultured in a BI-S-33 medium.²⁰ After three washes with phosphate-buffered saline (PBS), the trophozoites were suspended in a 1:10 PBS solution, sonicated, and centrifuged at 14,000 rpm for 30 minutes. The supernate was used as crude antigen to detect antibodies against *E. histolytica*.¹⁹

Methods. The DNA fragment-coding C terminus of an intermediate subunit of galactose- and N-acetyl-D-galactosamine-inhibitable lectin (C-Igl)^{21–24} was amplified by using PCR. The

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oligonucleotide primers used were¹⁹ EhIgl-S603: 5'-CCCTCGA GGAAGGACCAAATGCAGAAGAT-3' and EhIgl-AS1088: 5'-CCCTCGAGTTAAATGCCTTTAGCTCCATT-3'. The DNA fragment coding C-Igl was ligated into the expression vector pET19-b. The plasmid with a correct insert direction was transformed into *Escherichia coli* strain BL21 Star (DE3) pLysS and induced with 1 mM isopropylthiogalactoside for protein expression in Luria-Bertani medium. The recombinant C-Igl protein was harvested as an inclusion body and purified for detecting antibodies against *E. histolytica*.

The ELISA was performed with serum samples in 96-well flat-bottom microtiter plates (Greiner Bio One, Frickenhausen, Germany) in duplicate. The wells of the ELISA plates containing 5 µg of crude antigen or 100 ng of recombinant C-Igl diluted with coating buffer (0.01 M Na₂CO₃, 0.035 M NaHCO₃, pH 9.6), were incubated overnight at 4°C in a moist chamber. The plates were washed with PBS-Tween and then treated with PBS containing 1% skimmed milk for 1 hour. A total of 100 µL of serum samples diluted to 1:400 with PBS were added to the wells and incubated for 1 hr at room temperature. After washing with PBS-Tween, the wells were incubated with 100 µL of horseradish peroxidase-conjugated goat anti-human IgG (diluted to 1:1,000 with 1% skimmed milk; MP Biomedicals, Santa Ana, CA) for 1 hour at room temperature. After subsequent washing with PBS-Tween, the wells were incubated with 200 µL of substrate solution (0.4 mg/mL of *o*-phenylenediamine in citric acid-phosphate buffer, pH 5.0, containing 0.001% hydrogen peroxide) for 30 minutes. The reaction was halted by the addition of 50 µL of 2.5 M H₂SO₄. The optical density at 490 nm was recorded by using an ELX800 universal microplate reader (Bio-Tek Instruments, Inc., Winooski, VT). The cut-off point for a positive result was defined as an optical density at 490 nm > 3 SD above the mean positivity rates for healthy controls.

Statistical analysis. Statistical analysis was performed by using SAS software version 9.1 (SAS Institute Inc., Cary, NC). The chi square test was first used to test the independence of sex, region, and age category for every 10 years of age. A logistic regression model with a general form was applied, and the probability (Pr) was calculated according to the equation

$$\Pr(y = 1|x_1, x_2, \dots, x_n) = \frac{e^{\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_n x_n}}{1 + e^{\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_n x_n}}$$

where $y = 1$ if the serum had a positive result in the C-Igl/crude antigen test, and $y = 0$ if the serum had a negative result.²⁵ The association between each variable and each outcome was explored by means of multivariate logistic regression modeling, with visual inspection acting as an auxiliary. The model building procedure incorporates the stepwise forward selection method. The main effect of variables that we were interested in remained in the model. High order effect of continuous variables and interaction terms were added according to the significance of likelihood ratio tests on the condition that marginality restrictions were observed to construct hierarchical models. Overall, a value $P < 0.05$ was considered statistical significant. Age was treated as a continuous variable in logistic regression models, and was mean centered to avoid multicollinearity caused by its high order effects. The C-Igl and crude antigen tests were regarded as two separate experimental tests. Thus, the same analysis was applied to both tests.

TABLE 1

Characteristics of patients tested for C-Igl and crude antigens of *Entamoeba histolytica* in China

Characteristic	No. tested	No. (%) positive for C-Igl	No. (%) positive for crude antigens	P*
Total	1,312	82 (6.25)	145 (11.05)	
Sex				NS (0.023)
M	678	36 (5.31)	62 (9.14)	
F	634	46 (7.26)	83 (13.09)	
Age, years				NS (0.002)
≤ 10	72	3 (4.17)	4 (5.56)	
11–20	154	5 (3.25)	16 (10.39)	
21–30	286	24 (8.39)	48 (16.78)	
31–40	318	25 (7.86)	42 (13.21)	
41–50	216	13 (6.02)	20 (9.26)	
51–60	148	9 (6.08)	9 (6.08)	
61–70	93	3 (3.23)	6 (6.45)	
> 70	25	0 (0)	0 (0)	
Province				< 0.001
Beijing	188	2 (1.05)	12 (6.38)	
Shanghai	130	5 (3.85)	9 (6.92)	
Sichuan	142	10 (7.04)	22 (15.49)	
Guangxi	189	6 (3.17)	18 (9.52)	
Guizhou	285	41 (14.39)	50 (17.54)	
Qinghai	190	1 (0.53)	5 (2.63)	
Sinkiang	188	17 (9.04)	29 (15.43)	

*Based on chi-square test for homogeneity. NS = not significant.

RESULTS

The ELISA reactivity to *E. histolytica* target antigens from residents of seven provinces in China is shown in Table 1. The age of the study participants ranged from 0 to 85 years (mean ± SD age = 36.14 ± 16.35 years). For 678 males and 634 females, mean ± SD age values were 35.83 ± 16.70 and 36.47 ± 15.98 years, respectively. In Table 1, the total positivity rate of the C-Igl test was 6.25% (5.31% for males and 7.26% for females). However, the difference between the values was not significant. Average C-Igl positivity rates of females and males in every age group and the mean C-Igl positivity rates of serum samples in the different provinces are also shown in Table 1.

The positivity rates of the crude antigen test were 11.05% for the total sample, 9.14% for the males, and 13.09% for the females. Sex, age, and province were all significant risk factors in the crude antigen test. In general, the samples from females 11–20 and 21–30 years of age in the provinces of Sichuan, Guizhou, and Sinkiang were more likely to be positive in the C-Igl and the crude antigen tests.

FIGURE 1. Mean positive seroprevalence rates for C-Igl of *Entamoeba histolytica* in China in each age group for males versus females.

FIGURE 2. Mean positive seroprevalence rates for crude antigen of *Entamoeba histolytica* in China in each age group for males versus females.

The age groups 21–30 and 31–40 years of age had the highest positivity rates, and age groups 41–50 and 51–60 years of age also had higher positivity rates than the other groups, but the differences in comparison of those of other age groups were not significant.

We grouped persons by an age of every 10 years and plotted the positivity rate against the midpoint age of each group for C-IgI (Figure 1) and for crude antigen (Figure 2). For males and females, the positivity rate of the C-IgI test increased with age until an age of 25 years and then decreased with age (Figure 1). A linear age effect is probably inadequate to capture this trend. Thus, it is reasonable to perform a formal statistical test for the quadratic term or even higher order effect for age. Meanwhile, at young ages, a higher positivity rate for C-IgI seems to be related to females compared with males. Nevertheless, the difference tends to decrease with age, and at ages > 50 years, the positivity rate for the C-IgI test was slightly higher in males than in females. This pattern suggests that an interaction might exist and prompts statistical testing on interaction terms between age and sex effects. Similar features justify testing a high-order age effect and interaction terms for crude antigen tests (Figure 2).

The stepwise model building procedure starts from the simplest model. The main effect of age, sex, and region remained in the model regardless of statistical significance because these three variables are of study interest. When the quadratic age effect was added, at one degree of freedom, the deviance

of model decreased 3.9432 for C-IgI and 6.5968 for crude antigen. Likelihood ratio tests suggest that the quadratic term of age has significant effect on C-IgI ($P = 0.047061$) and crude antigen ($P = 0.010216$). Furthermore, adding an age $\times$ sex interaction led to a reduction of deviance at one degree of freedom (4.2765 for C-IgI and 1.7491 for crude antigen). Likelihood ratio tests detected a significant age $\times$ sex interaction on C-IgI ($P = 0.038642$) but not on crude antigen ($P = 0.18599$). No interaction involving region or the sex gender $\times$ age $\times$ age interaction was shown to be significant by using likelihood ratio tests. The final model for C-IgI (Table 2) contains the quadratic age effect and age $\times$ sex interaction and the main effect of age, sex, and region. The final model for crude antigen (Table 2) includes the same effects except for the age $\times$ sex interaction. Hosmer and Lemeshow goodness-of-fit tests demonstrated that either model fits the data well, ($P = 0.9499$ for C-IgI and 0.8395 for crude antigen (Table 2, Supplemental Table 1, and Supplemental Table 2).

In the logistic regression model for the C-IgI outcome, the dependent variable was the binary variable (1 = positive and 0 = negative outcome for the C-IgI test). The independent variables included the binary variable sex (1 = female and 0 = male), mean-centered age, square of mean-centered age, the interaction term of sex and age, and the six-level categorical variable region. The probability of a positive outcome of C-IgI test is shown in Figure 3 and was calculated according to the equation

$$\Pr(\text{outcome} = 1)$$

$$= \frac{e^{-4.65 - 0.00091(\text{Age} - 36.14)^2 + 0.00664(\text{Age} - 36.14) + 0.3781\text{gender} - 0.0356(\text{Age} - 36.14) \cdot \text{Gender} + \beta_{\text{Region}}}}{1 + e^{-4.65 - 0.00091(\text{Age} - 36.14)^2 + 0.00664(\text{Age} - 36.14) + 0.3781\text{gender} - 0.0356(\text{Age} - 36.14) \cdot \text{Gender} + \beta_{\text{Region}}}}$$

where $\beta_{\text{Region}} = 1.29$ for Guangxi, 2.91 for Guizhou, -0.71 for Qinghai, 1.43 for Shanghai, 1.96 for Sichuan, 2.33 for Sinkiang, and 0 for Beijing.

For persons of the same sex and the same age, the probability of a positive outcome of the C-IgI test is significantly higher in Guizhou, Sinkiang, and Sichuan than in Beijing (odds ratios = 18.32, 10.28 and 7.13, respectively), but Guangxi, Qinghai, and Shanghai did not have significant difference compared with Beijing. The odds ratios and 95% confidence intervals are shown in Table 2.

A significant interaction between age and sex effect implies that the odds ratio of a positive result of the C-IgI test between

TABLE 2
Logistic regression analysis of seroprevalence of C-IgI and crude antigens of *Entamoeba histolytica* in China*

Characteristic	Coefficient $\pm$ SE	Odds ratio	95% Confidence interval	P
Intercept	-4.6511 ± 0.7301 (-2.8522 ± 0.3238)			< 0.0001 (< 0.0001)
Age $\times$ age	-0.00091 ± 0.000462 (-0.00083 ± 0.000346)			0.0482 (0.0158)
Age $\times$ sex	-0.0356 ± 0.0176			0.0431
Age	0.00664 ± 0.0124 (-0.0180 ± 0.00654)			0.5908 (0.0060)
Sex				
F vs. M	0.3781 ± 0.2421 (0.4487 ± 0.1840)	1.566	(1.092–2.247)	0.1184 (0.0147)
Region†				
Guangxi vs. Beijing	1.2904 ± 0.8294 (0.5881 ± 0.3960)	3.634 (1.800)	0.715–18.466 (0.829–3.912)	0.1197 (0.1375)
Guizhou vs. Beijing	2.9080 ± 0.7337 (1.2867 ± 0.3405)	18.319 (3.621)	4.349–77.160 (1.858–7.058)	< 0.0001 (0.0002)
Qinghai vs. Beijing	-0.7141 ± 1.2309 (-0.9269 ± 0.5453)	0.490 (0.396)	0.044–5.466 (0.136–1.152)	0.5618 (0.0892)
Shanghai vs. Beijing	1.4361 ± 0.8492 (0.2528 ± 0.4615)	4.204 (1.288)	0.796–22.208 (0.521–3.181)	0.0908 (0.5839)
Sichuan vs. Beijing	1.9645 ± 0.7854 (1.0418 ± 0.3817)	7.131 (2.834)	1.530–33.246 (1.341–5.989)	0.0124 (0.0063)
Sinkiang vs. Beijing	2.3298 ± 0.7574 (1.0952 ± 0.3643)	10.276 (2.990)	2.329–45.344 (1.464–6.105)	0.0021 (0.0026)

*Values in parentheses are those for crude antigens.

†Beijing was used as the referent.

FIGURE 3. Probability of positive C-IgI test result for *Entamoeba histolytica* in China predicted by the model used.

males and females varies with age. Thus, the odds ratio of sex must be estimated with the presence of age. The estimated log odds ratio of a female versus a male from the same region at the age of a years was $\ln[\text{odds ratio}(\text{sex} = 1 \text{ versus } \text{sex} = 0, \text{age} = a)] = 0.3781 - 0.0356a$. The estimator of variance was $\text{var}[\ln[\text{odds ratio}(\text{sex} = 1 \text{ versus } \text{sex} = 0, \text{age} = a)]] = 0.0586088 + 0.003089a^2 + 2a(-0.00155)$, where 0.0586088 is the variance of the sex coefficient, 0.03089 is the variance of age $\times$ sex interaction coefficient, and -0.00155 is the covariance of the age and age $\times$ sex term. By calculating the endpoints as

$$\ln[\text{odds ratio}(\text{sex} = 1 \text{ versus } \text{sex} = 0, \text{age} = a)] \pm z_{1-\alpha/2} SE$$

and then exponentiating them, the odds ratios and the 95% confidence intervals were obtained. Odds ratios and the 95% confidence intervals calculated for a female versus a male from the same region at the ages of 5, 10, 15, 20, ..., 80 years are shown in Table 3. From a younger age to 33 years of age, females have higher rates of positive results for the C-IgI test, and the difference was statistically significant (odds ratios = 1.63–4.42). Among persons > 33 years of age, the difference between males and females loses statistical significance with regard to the C-IgI test. When age is greater than a value between 45 and 50 years, the model suggests a higher probability of positive C-IgI results among males than among females from the same region. However, the difference lacked statistical significance.

Similarly, a logistic regression model using the outcome of the crude antigen test as the dependent variable was developed (Table 2). This model also included the square of adjusted age. The probability of a positive outcome for the crude antigen test, which is shown in Figure 4, was calculated by using the function obtained from the final logistic regression model

$$\Pr(\text{outcome} = 1)$$

$$= \frac{e^{-2.8522+0.4487\text{Gender}-0.018(\text{Age}-36.14)-0.00083(\text{Age}-36.14)^2+\beta_{\text{Region}}}}{1+e^{-2.8522+0.4487\text{Gender}-0.018(\text{Age}-36.14)-0.00083(\text{Age}-36.14)^2+\beta_{\text{Region}}}}$$

where β_{Region} = 0.5881 for Guangxi, 1.2867 for Guizhou, -0.9269 for Qinghai, 0.2528 for Shanghai, 1.0418 for Sichuan, 1.0952 for Sinkiang, and 0 for Beijing.

Comparing persons of the same sex and the same age showed that Guizhou, Sinkiang, and Sichuan differed significantly from Beijing (odds ratios = 3.62, 2.99, and 2.834, respectively). Among persons at the same age from the same region, the odds ratio of a female versus a male was 1.57 for a positive result for the crude antigen test ($P = 0.0147$). The 95% confidence intervals are shown in Table 2.

DISCUSSION

Although infection with *E. histolytica* has a worldwide distribution and is common in persons in tropical and subtropical areas of developing countries, it often results in serious medical and socioeconomic problems. However, this protozoan infection does not have a high prevalence in China,¹⁴ and all data for direct microscopy of fecal samples suggest that *E. histolytica* infection may be endemic to rural areas of China.^{26,27} Furthermore, the present prevalence of *E. histolytica* infection in China is not known.

From a public health perspective, obtaining epidemiologic data on *E. histolytica* infection in China is important. An

TABLE 3

Estimated odds ratio of females versus males for a positive result for C-IgI antigen of *Entamoeba histolytica* in China, controlling for age

Age, years	Odds ratio	SE	95% Confidence interval	P
5	4.4155	2.5800	1.4048–13.8786	0.0110
10	3.6965	1.8689	1.3722–9.9574	0.0097
15	3.0945	1.3320	1.3310–7.1942	0.0087
20	2.5905	0.9354	1.2765–5.2572	0.0084
25	2.1687	0.6540	1.2009–3.9164	0.0103
30	1.8155	0.4697	1.0933–3.0146	0.0212
35	1.5198	0.3673	0.9465–2.4406	0.0832
40	1.2723	0.3251	0.7711–2.0994	0.3459
45	1.0651	0.3153	0.5962–1.9027	0.8313
50	0.8917	0.3158	0.4454–1.7851	0.7461
55	0.7464	0.3155	0.3260–1.7092	0.4890
60	0.6249	0.3108	0.2358–1.6563	0.3445
65	0.5231	0.3012	0.1692–1.6169	0.2604
70	0.4379	0.2875	0.1209–1.5859	0.2085
75	0.3666	0.2709	0.0861–1.5604	0.1745
80	0.3069	0.2524	0.0612–1.5387	0.1510

FIGURE 4. Probability of positive crude antigen test result for *Entamoeba histolytica* in China predicted by model used.

overview on the prevalence of *E. histolytica* infection in China could help predict future effects of *E. histolytica* infection on health care resources, as well as provide opportunities for evaluating the interventions. The use of serologic data to estimate the prevalence of infection requires an antibody assay that is sensitive and specific for recent *E. histolytica* infections. If there were sequential serologic data for *E. histolytica* infections, the method described in the current study could be used to estimate the prevalence. Because *E. histolytica* is morphologically indistinguishable from *E. dispar*,^{28–31} an ELISA was used to investigate the seroprevalence of *E. histolytica* infections.

Several commercial kits using crude antigen are now available for serodiagnosis of amebiasis, and recombinant C-IgI has a higher specificity than that described in our previous study.¹⁹ Another advantage of the recombinant protein is that the use of defined proteins in serodiagnosis facilitates standardization of the assays. Moreover, production of large quantities of recombinant C-IgI may be an economically effective alternative to ameba trophozoite cultivation.³² Therefore, we strongly recommend the use of recombinant C-IgI rather than crude antigen in serodiagnosis.

In the present study, C-IgI, a recombinant antigen, was selected as the diagnostic antigen, in addition to crude antigen. We studied 1,312 serum samples from seven provinces in China. The positivity rates for C-IgI determined by using an ELISA were 1.06% (2 of 188) for Beijing, 3.85% (5 of 130) for Shanghai, 7.04% (10 of 142) for Sichuan, 3.17% (6 of 189) for Guangxi, 14.39% (41 of 285) for Guizhou, 0.53% (1 of 190) for Qinghai, and 9.04% (17 of 188) for Sinkiang. However, the prevalence rates of *E. histolytica*/*E. dispar* infection obtained using the direct microscopy of fecal samples in Beijing, Shanghai, Sichuan, Guangxi, Guizhou, Qinghai, and Sinkiang provinces were 0.29%, 0.008%, 0.84%, 1.5%, 2.25%, 0.48%, and 2.4%, respectively.¹⁴ Because microscopy has low sensitivity and specificity for detection of amebiasis, these prevalence rates might be underestimates.

Conversely, because the serosurvey detected a sustained antibody response that persisted even after the infection,^{33,34} it contributed to the higher prevalence of infection in the present study compared with that in the 1992 survey. The

seroprevalence we found in the current study provides an overview of *E. histolytica* infection in China, but does not provide information on the incidence of amebiasis. The ELISA using serum samples showed a relatively low positivity rate for *E. histolytica* infection in Beijing, Shanghai, and Qinghai. Such findings were reasonable for Beijing and Shanghai because of their better sanitation infrastructure and health education programs. In Qinghai Province, the low temperature and dry air are not suitable for survival of *E. histolytica* cysts.^{35,36} Higher positivity rates were obtained in Sichuan, Guizhou, and Sinkiang Provinces because of their hygiene and environmental conditions.

Higher seropositivity rates (Table 1) and lower odds ratios (Table 2) were observed when crude antigen was used. The seropositivity rate in ELISA using the crude antigen was higher than that in ELISA using C-IgI. This result can be attributed to the lower specificity of crude antigen, as reported.^{19,35}

The age groups 21–30, 31–40, 41–50 and 51–60 years had higher positivity rates, but the differences when compared with other age groups were not significant. This finding could be caused by their higher populations compared with those of other age groups, or by the fact that persons 21–60 years of age have more social activities and greater opportunity for exposure to *E. histolytica*. The results also indicated that females have higher seropositivity rates than males (Table 1), which is further represented in the odds ratios shown in Table 2. However, no statistical differences were detected. To our knowledge, there is no sex difference for intestinal amebiasis. However, amebic liver abscess is 3–10 times more common in males.³⁶ The reason for the higher seropositivity of the female population is unclear.

The logistic regression model indicates the presence of some residual spatial autocorrelations after accounting for covariate effects, indicating that the variables used in the models did not capture all spatial variations in the observations.^{37,38} The logistic regression model described in the present study facilitates generation of prevalence data through the novel application of likelihood-based estimation to the analysis of sequential serologic data without any clinical

information. The logistic regression model can be used to assess the infection probability of a serum sample, provided the age, sex, and province of the patient are known. However, without any information concerning clinical symptoms, related diseases, or individual customs, predicting the probability of a positive result on the basis of general background information alone is inappropriate. Therefore, the model could only be used for the analyses of a few risk factors. The potential use of this model for prevalence is not supported by the data from our study.

The use of serologic data to estimate the prevalence of infection requires an antibody assay that is sensitive and specific for recent infection. Otherwise, estimation of the infection rate by using clinical surveillance data would be difficult until epidemic peaks are observed. The logistic regression model in this report paper provides an improved tool for estimating prevalence. The model can be continuously optimized. For instance, if a new serum sample or updated background information of patients is obtained, some coefficients in the model can be modified by incorporating the information into the model, thereby making it more competent. The application of such methods to epidemiologic research on estimation of covariate effects and predictive mapping has been demonstrated in two studies.^{39,40} The logistic regression model created in the current study can be used widely in other epidemiologic surveys by simply changing the coefficients in the software. The current analysis provides a more rigorous analytical approach, potentially resulting in more accurate parameters and significant estimates, as well as increased predictive accuracy.⁴¹

In summary, gaining an overview of the seropositivity of *E. histolytica* infection in seven provinces of China, coupled with our logistic regression model estimation method, provide a more accurate measure of incidence and prior probability of the prevalence of infection, thereby facilitating better resource allocation to protect the population from public health concerns. The logistic regression model has general applicability to any sequential serologic data set obtained over a period of changing incidence.

Received October 8, 2011. Accepted for publication April 14, 2012.

Note: Supplemental tables appear at www.ajtmh.org.

Financial support: This study was supported by grants from the Major Project of the Eleventh Five-Year Plan (No. 2009ZX10004104), the National Science Foundation of China (No. 30771878 and No. 81771594), and Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 23117009 and No. 24406013).

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