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Epidemiological Study of Amebiasis and Strain Analysis of Pathogenic Amoeba in an Education and Nursing Institute for the Mentally-Handicapped in Taiwan

Abstract

To appreciate the prevalence of intestinal amoebic infection in a public education and nursing institute for the mentally-handicapped in Taiwan, screening for intestinal amoeba with enzyme immunoassay (EIA) and direct microscopy were performed. The species of amoeba were then confirmed using the polymerase chain reaction (PCR). The extent, sources, and risk factors of infection were analyzed with epidemiological studies.

The results showed that there was no EIA positive reaction among 182 employees, but 38 (8.6%) of the 442 residents were EIA positive. Cysts and trophozoites of *Entamoeba histolytica* and/or *E. dispar* were detected in all EIA positive stool specimens. 15 (39.5%) of the residents were infected with *E. histolytica*, and 23 (60.5%) were infected with *E. dispar*. All infected residents were asymptomatic. In addition, the amoeba serum antibody tests of the residents were 44.1% (195/442) positive (IHA titer≧1:256X). There was a

positive correlation between the severity of mental retardation and the distribution of serum-positive residents.

As to the risk factors of the infection: when PCR results positive for *E. histolytica* or positive amoeba serum antibody tests (IHA titer≧1:256X) were selected as indices of amebiasis, the odds ratio was 3.89 (95 % Confidence Interval 0.95～22.63) and 1.79 (95 % Confidence Interval 0.94～3.41) among infected and non-infected groups for drinking unboiled tap water and abnormal behavior, respectively. The statistical significance was marginal (p value= 0.0611 and 0.0558, respectively).

Keywords: Amebiasis, PCR, Education and Nursing Institute for the Mentally-handicapped

Introduction

The pathogen of the notifiable disease amebiasis is the parasitic protozoa *Entamoeba histolytica*. According to the WHO, more than five hundred million people around the world have amebiasis. Thirty-six million of these have amebic colitis or extra-intestinal abscesses, and eighty thousand have died of the disease. The mortality rate was ranked No. 3 among parasitic infections [1]. The life cycle of *E. histolytica* has trophozoite and cyst stages. The former cannot survive independently, and the latter is infectious and hardy, surviving in harsh environments. *E. histolytica* is transmitted via the fecal-oral route. The incubation period maybe last from days to years (average duration is two to four weeks) [2]. The majority of those infected (90%) are asymptomatic carriers, and *E. histolytica* resides in the intestinal tract in symbiosis with the host. Few of the infected have GI symptoms such as diarrhea, or manifestations of enterocolitis.

In 2-20% of cases, the protozoa may invade extra-intestinally and form abscesses. The most common extra-intestinal manifestation is liver abscess [3].

Identification of cases is the key to disease prevention, and accurate tests are of vital importance. Traditionally, intestinal amoeba infection is diagnosed by direct microscopic examination for trophozoites or cysts and morphological identification. Haematophagous amoeba trophozoites is the diagnostic criteria for invasive amebiasis [4]. Recently, *E. histolytica* was classified into two species, *E. histolytica* and *E. dispar* according to biochemical, immunological, and genetic evidences. *E. histolytica* is the invasive pathogen in amebiasis, while *E. dispar* is a symbiotic intestinal protozoa. They are microscopically indistinguishable [5]. In 1997, the WHO/PAHO/UNESCO decided to revise the case definition of amebiasis: the term *Amebiasis* now being applied to symptomatic and/or asymptomatic *E. histolytica* infection. They also suggested that therapy should be withheld until the species of amoeba has been identified [6]. Because of the low sensitivity of microscopic examination, and potential interference with WBCs and other non-pathogenic protozoa in stool, other diagnostic methods, such as EIA for specific *E. histolytica* antigen, molecular detection of *E. histolytica* DNA fragments [7,8], and IHA detection of *E. histolytica*-specific antibody in serum, are required. These methods have their own pros and cons. EIA is sensitive, easy to perform, but cannot distinguish between *E. histolytica* and *E. dispar*. The Molecular detection method has the sensitivity of EIA, can distinguish the two species, but is technically demanding and labor intensive. IHA can assist in the diagnosis of amebiasis, but cannot distinguish past and ongoing infections. Because there is a 2.2% sequence difference in small subunit ribosomal RNA gene (SSU-rDNA) between *E. histolytica* and *E. dispar*, specific primers can be designed, and polymerase chain

reaction (PCR) can diagnose both of them simultaneously [9, 10].

In Taiwan, *E. histolytica* mainly infects patients in institutes of psychiatric rehabilitation, residents in educational/nursing institutes for the mentally-handicapped, individuals returning from epidemic areas, alien workers, alien wives, male homosexuals, and residents of remote districts. Due to communal living and abnormal behavior, patients in institutes of psychiatric rehabilitation and residents in educational/nursing institutes for the mentally-handicapped are high risk groups for amebiasis. Between 1987 and 1990, the seropositive rate for amebiasis among 4,803 patients of 12 institutes of psychiatric rehabilitation in Taiwan was almost 30% (IHA). The highest rate (45.39%) was in an eastern mental hospital. In 1994 when screening for parasitic infection was performed in that hospital, the positive rate of amebiasis by microscopic examination was 10.9% [11]. In educational/nursing institutes for the mentally-handicapped, sporadic cases of liver abscess and amebiasis occasionally occur. The seropositive rate is between 13.1% and 29%, and the microscopic positive rate is between 0.001% and 15.2% [12, 13]. Because most infections are asymptomatic, it is impossible to identify potential invasive amebiasis or carriers by stool microscopic examination for haematophagous trophozoites or HIA. In addition, because previous epidemiological studies of intestinal amebiasis depended on microscopic examination and non-specific clinical symptoms for diagnosis, the prevalence of amebiasis was overestimated, and the real target of disease prevention, the carriers, could not be identified.

A southern Taiwan Medical Center reported a case of amoebic dysentery in January 2001. The patient who came from a public educational institute for the mentally-handicapped, was admitted because of diarrhea, fever and vomiting. Invasive intestinal amebiasis was then diagnosed using the serological method.

Stool specimens were negative for the protozoa. In the same month, the local public health bureau screened close contacts in the same institute, and found 9 of 67 close contacts (49 residents, 11 assistants, 7 cooks) to be infected with amoeba, diagnosed using direct microscopic examination. All of them were asymptomatic. To appreciate the prevalence of intestinal amebiasis in that institute, we used EIA along with direct microscopic examination to screen for intestinal amebiasis in the whole institute, and the molecular method to identify the species of protozoa. The extent, source, and risk factors of infection were investigated using epidemiological studies.

Materials and Methods

Introduction to the Institute

The institute, with 182 employees, is located in a rural area of southern Taiwan; the institute is a special public educational facility for patients with mild to severe mental retardation and multiple disabilities. 442 residents lived in 10 buildings. The tenth building, in which 16 residents lived, was located near the campus. Each of the remaining nine buildings had an average of 47 residents. The third and forth buildings were for female. Every building had four bedrooms, two consultant rooms, one restroom, one bathroom, and one central living room. There were two water systems in the building: tap-water for drinking and cooking, and groundwater for washing, hygiene, and other usage. The groundwater used was precipitated, filtered, aerated, and chlorinated. Foods were processed by cooks. Residents ate their meals in a communal room.

Subjects of Study

The subjects of this study were 442 residents in the institute, 346 male

(78.3%) and 96 female (21.7%). Their age range was 20 to 68, the average age was 36, and the median was 29. 3 residents (0.7%) had mild mental retardation, 38 (8.6%) moderate, 116 (26.2%) severe, and 285 (64.5%) very severe. In addition, 104 of them had multiple disabilities (limb and visual or hearing impairment). All employees of the institute were also subjected to screening for amebiasis.

Intestinal Amoeba Screening

The subjects of screening included all employees and residents in the institute. After collection, the stool specimens were stored at 4℃ and subjected to processing or analysis within eight hours. All specimens were analyzed with ProSpect® *Entamoeba histolytica* Microplate Assay (Alexon, USA) kits. Stool specimens of EIA positive cases were then fixed and stained with MIF or fixed with PVA and stained with trichrome, and confirmed microscopically. After pre-treatment, microscopically positive specimens were transferred to the parasitology laboratory of the CDC Research and Laboratory Center to identify species of amoeba with PCR.

Identification of Amoeba Species

Stool DNA Extraction

DNA of amoeba cysts or trophozoites was extracted using the method previously described [14]. Fresh stool specimens were mixed with guanidine thiocyanate and then centrifuged. 10% NP-40 was added to the supernatant, and DNA was extracted with Celite®. Extracted DNA was then rinsed with ethanol and acetone, and released with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Celite® was removed by centrifugation. The DNA was then subjected to

species identification or stored at -20 ℃.

Primer Design

The primers were designed based on *E. histolytica* and *E. dispar* SSU-rDNA in GenBank data. The index numbers and base pairs of DNA were gi 9283/emb X56991 (1947 bp) for *E. histolytica* and gi 1212896/emb Z49256 (1949 bp) for *E. dispar*. GCG system (Genetic Computer Group package) were used for comparison and design. Design for nested and two step method were used to increase sensitivity. Selective adhesion was on the 3' ends of the primers for species identification. The specificity of the primers was confirmed with BLAST [15] search and comparison in the GenBank and in PCR products. The sequence comparison between *E. histolytica* and *E. dispar* SSU-rDNA and location of the primers was shown in reference 16.

Polymerase Chain Reaction

In the first step, Outer1 (5'-GAA ATT CAG ATG TAC AAA GA-3')/Outer 1R (5'- CAG AAT CCT AGA ATT TCA C-3') and UidA1 (5'- AGA TAT TCG TAA TTA TGT GG-3') /UidA2 (5'-AGA AAT CAT GGA AGT AAG AC-3') primer pairs were used. The former can direct the amplification of a 823-bp product in the SSU-rDNA of the two species, and the later can amplify 320-bp in the uidA gene of *Escherichia coli* as the positive control. We used 5 l DNA templates, 0.5 M Outer1/Outer1R and UidA1/UidA2, PCR buffer (10 mM Tris-HCl, pH8.3, 50 mM KCl), 200 M dNTP, 1.5 mM $MgCl_2$, 2%(w/v)sucrose, 0.1 mM Cresol Red, 0.1 g/ l BSA, 0.05 U/ l AmpliTaq® DNA polymerase (Applied Biosystems, USA) in this reaction. The total volume was 50 l. The reaction proceeded in a GeneAmp PCR 9700 (Applied Biosystems, CA, USA), and the parameters were: 94 ℃ for 2 min, then 35 cycles at 94 ℃ for 15 sec, 47

℃ for 15 sec, and 72 ℃ for 1 min, and followed by 72 ℃ for 6 min to terminate the reaction. In the second PCR reaction, we used Eh1 (5'- AAG CAT TGT TTC TAG ATC TG-3')/Eh2 (5'- CAC GTT AAA AGA GGT CTA AC-3') and Ed1 (5'- AAA CAT TGT TTC TAA ATC CA-3') /Ed2 (5'- ACC ACT TAC TAT CCC TAC C-3') primer pairs. The former can amplify 447-bp of the *E. histolytica* SSU-rDNA, and the latter can amplify 603-bp in the *E. dispar* SSU-rDNA. In this reaction, we used 2.5μl of product of the first reaction, 0.5μM Eh1/Eh2 and Ed1/Ed2, PCR buffer, 200μM dNTP, 1.5 mM $MgCl_2$, 2% （w/v）Sucrose, 0.1 mM Cresol Red, 0.1μg/μl BSA, and 0.05 U/μl AmpliTaq® DNA polymerase. The total reaction volume was 25 l. After 2 min at 94 ℃, 35 reaction cycles proceeded. They were: 94 ℃ for 15 sec, 52 ℃ 15 for sec, 72 ℃ for 40 sec, and followed by 72 ℃ for 6 min to terminate the reaction. The PCR products were then fractionated in 3% agarose gel (3:1 Nusieve agarose gel), stained with 0.5μg/ml ethidium bromide, and visualized under UV light [16].

Serum Anti-amoeba Antibody Analysis

We used the IHA method for analysis. Taking into consideration the physical examination data of the residents in 1995, 1999, 2000 and 2001, we used titer equal or more than 1:256X as positive.

Questionnaire

A questionnaire was filled out while screening was performed. A semi-structured questionnaire was used. Basic profiles of cases, admission date, severity of mental retardation, GI symptoms, date of disease onset, degree/kind of medical care, and habits of personal hygiene were investigated. The subjects were residents and employees of the institute. Because the majority of the

residents suffered from various degrees of mental retardation and they could not fill out the questionnaire independently, assistance by nurses, guardians, or consultants was required.

Data Processing and Analysis

Data from questionnaires was processed with EPI-info 6.0 [17], debugged, and analyzed. Variants were tested with Chi-square test and p less than 0.05 was set for statistical significance. Positive results of *E. histolytica* infection or seropositivity for anti-amoeba antibody were used as index of *E. histolytica* infection. All residents were divided into infected or non-infected groups. Each variant or risk factor related to *E. histolytica* infection was shown by odds ratio and 95% confidence interval (CI). The odds ratio was statistically significant if its 95% CI did not include 1.00.

Results

The EIA results showed that none of the 182 employees in the institute had positive results, while 38 of the 442 residents (8.6%) showed positive reactions. All EIA positive residents had cysts or trophozoites of *E. histolytica*/ *dispar* under microscopic examination. 81.6% (31/38) of them were infected by cysts, 5.3% (2/38) were infected by trophozoites, and 13.2% (5/38) had mixed infection. The trophozoites did not phagocyte RBC under each microscopic examination. 19 of the EIA positive cases had other intestinal protozoa in their stool specimens. 6 residents had *Blastocystis hominis*, 1 had *E. hartmanni*, 5 had *B. hominis* and *Endolimax nana*, 1 had *B. hominis* and *E. coli*, 1 had *B. hominis* and *E. hartmanni*, 1 had *E. hartmanni* and *Endolimax nana*, 1 had *E. coli* and *Endolimax nana*, 2 had *E. hartmanni*, *B. hominis*, and *Endolimax nana*, and 1 had *E. hartmanni*, *B. hominis*, and *E. coli*. None of them had the ova of helminths.

Statistically significant (p value<0.05) differences in cases positive for *E. histolytica*/*E. dispar* included female gender and residents less than 10 years of age. There were no difference in categories of age, severity of mental retardation, and multiple disabilities (Table 1).

The results of species identification with PCR and comparison with that of microscopic examination were shown in Table 2. Most of us suspected that trophozoites would more easily be identified in soft stools or specimens of diarrhea, but there was no direct relationship between cyst or trophozoites identified under microscope and infection caused by *E. histolytica* or *E. dispar*.

Table 3 showed that the *E. histolytica* positive residents living quarters were distributed in the first, second, third, fourth, fifth and seventh buildings. The positive rates were between 2.0% and 17.0%, and the highest was from the residents of the fourth building. *E. dispar* positive residents resided in the first, second, third, fifth, sixth, seventh and eighth buildings. The positive rates were between 2.1% and 12.2%, and the highest was from the third building. In summary, among the 38 microscopically positive cases, 39.5% (15/38) had *E. histolytica*. The prevalence rate was 3.4% (15/422). 60.5% (23/38) were positive for *E. dispar*, and the prevalence rate was 5.2% (23/442). There were no mixed infections of *E. histolytica* and *E. dispar*. The 15 *E. histolytica* positive cases showed no clinical symptoms on the questionnaires or in the medical history. 14 of them had an IHA titer equal to or more than 1:256X.

The anti-amoeba antibody positive (IHA titer≧1:256X) rate in the residents was 44.1% (195/442). There was a positive and statistically significant (P value< 0.05) correlation between the distribution of seropositive cases and the severity of mental retardation, and there was no difference in categories of age, gender, multiple disabilities, year of residency (Table 4). The distribution of

seropositive cases is shown in Table 5. All buildings had seropositive cases, and the rates were between 8.5% and 63.8%, the highest rate being from the fourth building (63.8%), and the lowest, from the ninth one (8.5%).

To study the risk factors for *E. histolytica* infection, 624 questionnaires were used, including 442 for residents and 182 for employees. The retrieval rate was 100% from residents, and 80% (145/185) from employees. Because there was no amoeba infection among employees, and none of them were seropositive for anti-amoeba antibody, the analysis of questionnaires focused mainly on the residents. If microscopically positive result for *E. histolytica* and IHA titer≧1:256X were used as indices of *E. histolytica* infection, all the residents were divided into infected and non-infected groups. There were 196 (146 male and 50 female) in the infected group. These two groups showed no difference in gender and age distribution. The risk factors for *E. histolytica* infection were shown in Table 6. It illustrates that there were no statistically significant (95% confidence interval all included 1.00) differences between these two groups in washing hands before meals, self-feeding, brushing teeth or rinsing of mouth at the sinks, face washing at common sinks, self sufficiency in stooling, washing hands after using the rest room, and assisting others in hygiene. The odds ratios were 3.89 (95% confidence interval 0.95~22.63) and 1.79 (95% confidence interval 0.94~3.41) respectively in drinking tap water from sinks and abnormal behavior between infected and non-infected groups. The statistical significance was marginal (p value= 0.0611 and 0.0558 respectively).

Discussion

In this study, we showed that in the institute, the positive rate for *E. histolytica* was 3.4% (15/442) (Table 3). All the positive cases were

asymptomatic, and 86.7% (13/15) had infective cysts in stool specimens. Therefore, according to WHO criteria, the prevalence rate of amebiasis was 3.4%. Nevertheless, the sensitivity of primary screening agents was merely 78% [18]. Hence, the prevalence of amoeba infection should be more than 3.4%.

The majority of *E. histolytica/dispar* positive cases were female with less than 10 years of residence. It is possible that this is because female residents resided in the institute between two and nine years with a median of eight years. There was a positive correlation between seropositivity (IHA titer≧1:256X) and the severity of mental retardation, and there was no relationship to gender. *E. histolytica*/*dispar* infection was higher in residents of less than 10 years. In follow-up studies for *E. histolytica dispar* asymptomatic carriers, the carrier status could last from several months to one year, and the majority of them would revert to non-carrier status spontaneously [19, 20]. In a retrospective epidemiological study of *E. histolytica* infection between 1929 and 1997, Acuña-Soto et al showed that there was no difference in distribution in *E. histolytica* infection between genders [21].

Amebiasis can be caused by ingesting infective cysts in contaminated water or foods, and by direct fecal-oral transmission. In institutions for psychiatric patients or the mentally handicapped, the risk of infection will be higher because of abnormal behavior [22, 23]. Meals in this institute were prepared by professional cooks, and screening for intestinal amebiasis and serological studies of employees showed no amoeba infection. Therefore, intestinal amoeba infection in this institute was not related to food and drinking water. To appreciate the risk factors of *E. histolytica* infection, questionnaires were used to study the relationship between personal hygiene habits and *E. histolytica* infection. The results showed that the odds ratios were 3.89 (95% confidence interval

0.95~22.63) and 1.79 (95% confidence interval 0.94~3.41) respectively in drinking tap water from sinks and abnormal behavior (garbage gathering, picking up food from the ground, playing with or ingesting stools) between infected and non-infected groups. The statistical significances were marginal (p value= 0.0611 and 0.0558 respectively). Because only a small proportion of residents drank tap water from sinks (12/442), the correlation of this behavior with *E. histolytica* infection cannot be established. Moreover, there was a positive and statistically significant (p value< 0.05) correlation between seropositivity and the severity of mental retardation. The more severe the mental retardation associated with abnormal behavior (garbage gathering, picking up food from the ground, playing with or eating stools), the more chance they had *E. histolytica* infection. Hygiene training should be intensified to reduce the chance of infection. Questionnaires in this study were filled by caregivers, because the residents all had varying degrees of mental retardation, and therefore, the answers were subject to recall and perception bias of the caregiver.

There was a fatal case of liver abscess in this institute in 1994 [12]. The rate of seropositivity of anti-amoeba antibody increased from 15.8% in 1995 to 44.1% in 2001, the positive rates were from 8.5% to 63.8% in the buildings (Table 3). Therefore, *E. histolytica* infection had been prevalent in the institute for a long time. According to the study of Gathiram, in southern Africa Durban, asymptomatic carriers of *E. histolytica* were all strongly positive serologically. In the one year follow-up, 10% (2/20) had amoeba colitis, and the rest of them were still asymptomatic with spontaneous remission in that period [24]. In 1997, WHO suggested the adoption of immunological or molecular methods for microscopically positive cases of *E. histolytica* and *E. dispar* before initiating treatment. Because most *E. histolytica* infections are asymptomatic,

asymptomatic carriers are the source of transmission of this disease. Screening, identification and radical treatment for asymptomatic carriers are necessary to eradicate the disease. In this study, *E. histolytica* and *E. dispar* infection coexisted in the institute, differing from Japanese institutes where *E. histolytica* infection was predominant [25]. Therefore, a differential diagnosis of *E. histolytica* and *E. dispar* infection is important in the prevention of *E. histolytica* infection in institutes. Stool EIA screening assisted by PCR species identification can accurately identify asymptomatic carriers for antibiotic treatment. Hence colitis and fatal liver abscess after asymptomatic infection of *E. histolytica* can be reduced. Side effects of unnecessary treatment and drug resistant stains can also be avoided. To prevent the disease, the entire institute should be screened periodically for intestinal *E. histolytica* infection, and asymptomatic carriers should be treated.

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References

1. Guerrant, RL: Amebiasis: introduction, current status, and research questions. Rev Infect Dis 1986; 8:218-227.

2. Anonymous: Amoebiasis. In: Chin J. ed. Control of Communicable Diseases Manual. 17th ed. American Public Health Association, Washington, DC. 2000; pp 11-15.

3. Bruckner D: Amebiasis. Clin Microbiol Rev 1992; 5: 356-369.

4. Ong SJ：The diagnosis and prevention of amoebiasis. Epi Bull 1994; 10:304-308. (Chinese)

5. Diamond LS, Clark CG.: A redescription of *Entamoeba histolytica* Schaudinn, 1903 (Emended Walker, 1911) separating it from *Entamoeba dispar* Brumpt, 1925. J Euk Microbiol 1993;40: 340-344.

6. Anonymous: WHO/PAHO/UNESCO report. A consultation with experts on amoebiasis. Epidemiol Bulletin 1997; 18:13-14.

7. Haque R, Kress K, Wood S, et al: Diagnosis of pathogenic *Entamoeba histolytica* infection using a stool ELISA based on monoclonal antibodies to the galactose-specific adhesin. J Infect Dis 1993;167: 247-249.

8. Weiss JB: DNA probes and PCR for diagnosis of parasitic infections. Clin Microbiol Rev 1995; 8:113-130.

9. Clark CG, Diamond LS: Ribosomal RNA genes of 'pathogenic' and 'nonpathogenic' *Entamoeba histolytica* are distinct. Mol Biochem Parasitol 1991;49: 297-302.

10. González-Ruiz A, Wright SG.: *Dispar*ate amoebae (editorial). Lancet 1998;351: 1672-1673.

11. Ong SJ, Cheng MY, Liu KH et al: The status of parasites infection in a Mental Hospita-with emphasis on the amebiasis. Epi Bull 1995; 11:179-183. (Chinese)

12. Chao DY, Wu PH, Chen KT et al: The study of amebic infection in a provincial education and nursing institute. Epi Bull 1997; 13:135-144.

(Chinese)

13. Jiang DS, Chang KH: An investigation of amebiasis outbreak in one rehabilitation center for mentally retarded children. Public Health 2000; 26:261-270. (Chinese)
14. Hung CC, Deng HY, Hsiao WH, et al: Invasive amebiasis as an emerging parasitic disease in patients with human immunodeficiency virus type 1 infection in Taiwan. Arch Intern Med 2005; 165:409-415.
15. Altschul SF, Madden TL, Schaffer AA, et al: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl Acids Res 1997; 25: 3389-3402.
16. Hsiao WH, JiangJS, Chan YH, et al: The Prevalence Survey of Amebic Infection at One Institution for the Mentally Retarded in Taiwan Using a Nested Multiplex PCR. (submitted)
17. Dean AG, Dean JA, Coulombier D, et al: Epi Info, Version 6.04a, a word processing, database, and statistics program for public health on IBM-compatible microcomputers. Atlanta: Centers for Disease Control and Prevention. 1996
18. Ong SJ, Cheng MY, Liu KH, et al: Use of ProSpecT® microplate enzyme immunoassay for the detection of pathogenic and non-pathogenic *Entamoeba histolytica* in faecal specimens. Trans R Soc Trop Med Hyg 1996; 90: 248-249.
19. Nanda R, Baveja U, Anand BS:*Entamoeba histolytica* cyst passers: clinical features and outcome in untreated subjects. Lancet 1984; 2: 301-303.
20. Anand BS, Tuteja AK, Kaur M, et al: *Entamoeba histolytica* cyst passers: clinical profile and spontaneous eradication of infection. Dig Dis Sci 1993; 38: 1825-1830.

21. Acuña-Soto R, Maguire JH, Wirth DF: Gender distribution in asymptomatic and invasive amebiasis. Am J Gastroenterol 2000; 98: 1277-1283.

22. Nagakura K, Tachibana H, Tanaka T, et al: An outbreak of amebiasis in an institution for the mentally retarded in Japan. Jpn J Med Sci Biol 1989; 42:63-76.

23. Nagakura, K., Tachibana, H., Kaneda, Y., et al: Amebiasis in institutions for the mentally retarded in Kanagawa Prefecture, Japan. Jpn J Med Sci Biol 1990; 43:123-131.

24. Gathiram V, Jackson TF: A longitudinal study of asymptomatic carriers of pathogenic zymodemes of *Entamoeba histolytica*. S Afr Med J 1987; 72: 669-672.

25. Tachibana H, Kobayashi S, Nagakura K, et al: Asymptomatic cyst passers of *Entamoeba histolytica* but not *Entamoeba dispar* in institutions for the mentally retarded in Japan. Parasitol Int 2000; 49: 31-35.

Table 1. Comparison of variables among amoeba-positive residents in the institute

Variables	Total	Positive cases	Positive rate	$\chi 2$	P
Gender[#]					
Male	346	23	6.6%	7.71	0.005
Female	96	15	15.6%		
Age					
<=40	245	24	10.6%	1.01	0.32
>40	197	14	6.5%		
Severity of mental retardation					
Mild to moderate	41	4	9.8%	0.6	0.74
Severe	116	8	6.9%		
Very severe	285	26	9.1%		
Multiple disabilities					
With	104	8	7.7%	0.14	0.71
Without	338	30	8.9%		
Years of residence[*]					
<=10	217	25	11.5%	4.64	0.031
>10	225	13	5.8%		
Total	442	38	8.6%		

[#][*] $p < 0.05$

※This result was obtained with EIA method.

Table 2. Species identification of amoeba with PCR method or direct microscopic examination

Species / Stage	*E. histolytica* Case Number (%)	*E. dispar* Case number (%)	Sum
Cyst	10 (66.7)	21 (91.3)	31
Trophozoite	2 (13.3)	0 (2.0)	2
Both	3 (20.0)	2 (8.7)	5
Sum	15 (100)	23 (100)	38

Table 3. Distribution of amoeba-positive cases among buildings

		E. histolytica		*E. dispar*	
Building	Cases screened	Positive cases	Positive rate	Positive cases	Positive rate
1	47	1	2.1%	4	8.5%
2	47	1	2.1%	2	4.3%
3	49	1	2.0%	6	12.2%
4	47	8	17.0%	0	0.0%
5	48	1	2.1%	1	2.1%
6	48	0	0.0%	5	10.4%
7	46	3	6.5%	4	8.7%
8	47	0	0.0%	1	2.1%
9	47	0	0.0%	0	0.0%
10	16	0	0.0%	0	0.0%
Sum	442	15	3.4%	23	5.2%

※The results were obtained with EIA method.

Table 4. Comparison of variables of seropositive residents

Variables	Total	Positive cases	Percentage	χ^2	P#
Gender					
Male	346	146	42.2%	2.38	0.12
Female	96	49	51.0%		
Age					
<=40	245	112	49.6%	0.57	0.45
>40	197	83	38.4%		
Severity of metal retardation					
Mild to moderate	41	12	29.3%	8.9	0.01
Severe	116	43	37.1%		
Very severe	285	140	49.1%		
Multiple disabilities					
With	104	45	43.3%	0.04	0.84
Without	338	150	44.4%		
Years of residence					
<=10	217	88	40.6%	2.2	0.14
>10	225	107	47.6%		
Total	442	195	44.1%		

*IHA titer≧1:256X for EIA positive。

#$p < 0.05$

※This results were obtained with PCR method.

Table 5. Distribution of seropositive resident among buildings

Buildings	No. Residents	Positive cases	Positive rate
1	47	23	48.9%
2	47	19	40.4%
3	49	19	38.8%
4	47	30	63.8%
5	48	25	52.1%
6	48	20	41.7%
7	46	26	56.5%
8	47	26	55.3%
9	47	4	8.5%
10	16	3	18.8%
Total	442	195	44.1%

※This results were obtained with PCR method.

Table 6. Risk factors of *E. histolytica* infection in residents

Risk factors	Infected group	Non-infected group	Odds ratio (95% confidence interval)
Age			
Average	35.5	36.2	
Range	20～68	20～68	
Gender			
Male	146	200	0.67 (0.41～1.09)
Female	50	46	
Washing hands before meals			
Often	186	232	1.12 (0.45～2.81)
Rare	10	14	
Self feeding			
Yes	179	215	1.52 (0.78～2.99)
No	17	31	
Brushing teeth or rinsing mouth at sinks			
Often	124	173	0.73 (0.48～1.11)
Rare	72	73	
Washing faces at sinks			
Often	142	187	0.83 (0.53～1.31)
Rare	54	59	
Drinking water at sinks[#]			
Yes	9	3	3.89 (0.95～22.63)
No	187	243	
Ability to handle stools			
Yes	131	173	0.85 (0.55～1.31)
No	65	73	
Washing hands after using rest room			
Often	170	218	0.81 (0.44～1.50)
Rare	26	27	
Assistant others for hygiene			
Yes	25	29	1.09 (0.59～2.02)
No	171	217	
Abnormal behaviors[*]			
Yes	28	21	1.79 (0.94～3.41)
No	168	225	
Total	196	246	

[#]p value＝0.0611； [*]p value＝0.0558, statistically marginally significant