Japan. J. Med. Sci. Biol., 42, 63 - 76, 1989.

AN OUTBREAK OF AMEBIASIS IN AN INSTITUTION FOR THE MENTALLY RETARDED IN JAPAN

Kouichi NAGAKURA, Hiroshi TACHIBANA, Tomoo TANAKA,
Yoshimasa KANEDA, Masahito TOKUNAGA¹, Masanaga SASAO²
and Tsutomu TAKEUCHI³

Department of Parasitology and ¹Department of Internal Medicine, School of Medicine, Tokai University, Bohseidai, Isehara, Kanagawa 259-11, ²Sasao Clinic, Minamiyana, Hadano, Kanagawa 257, and ³Department of Parasitology, School of Medicine, Keio University, Shinanomachi, Shinjuku-ku, Tokyo 160

(Received April 28, 1989. Accepted October 6, 1989)

SUMMARY: The results of an epidemiological survey in a 190-patient institution for mentally retarded were reported. Twenty percent of the patients had either cysts or trophozoites of *Entamoeba histolytica* in their stools, and 38% were positive serologically. The amebic outbreak revealed a sex-independent but age-dependent distribution; younger patients had more serious symptoms in cases invasive amebiasis. A high prevalence of amebic infection was found in the heavily retarded patients, and the positive cases tended to concentrate in certain training classes. Further demographic analysis suggests that the amebic infection was possibly caused by abnormal behavior of heavily retarded patients.

INTRODUCTION

Although traditionally considered a disease common only in less developed countries, amebiasis due to *Entamoeba histolytica* has been recognized recently

永倉貢一・橘 裕司・田中朝雄・金田良雄(東海大学医学部寄生虫学教室 神奈川県伊勢原市望星台)

徳永雅人(東海大学医学部第四内科 神奈川県伊勢原市望星台)

笹尾昌永(笹尾内科医院 神奈川県秦野市南矢名)

竹内 勤(慶応大学医学部寄生虫学教室 東京都新宿区信濃町35)

as an urban-type infection. Most cases used to be considered an exotic illness related to foreign travel or to contaminated food or water. In Japan, the incidence of amebiasis decreased since the World War II. From the end of the 1970s, however, it suddenly started to increase. This was attributable primarily to sexual transmission (1). The endemic foci of amebiasis and the presence of a high risk group for this enteric infection other than male homosexuals have scarcely been reported.

The importance of person-to-person spread of this protozoa is now generally acknowledged (2,3). In sexually transmitted amebiasis, homosexuality and oral-anal sex are important risk factors for *E. histolytica* infection (3). In addition to sexually transmitted amebiasis, the direct person-to-person spread of ameba is more likely in institutions for the mentally retarded (4). In such mental institutions, the incidence of stools positive for cysts or trophozoites of *E. histolytica* ranged from 0% to 71% (4) and high rates of seropositivity (14% and 28%) have been found (5,6). Demographic data from these studies and many personal observations have suggested person-to-person transmission, but no direct evidence has been available.

In April, 1986, we diagnosed a liver abscess patient as invasive amebiasis in an institution for the mentally retarded in Kanagawa Prefecture, Japan. Several surveys showed the highest prevalence of amebic infection in the institution. The purpose of this investigation was to establish the epidemiological background of outbreaks of amebic infection among the mentally retarded patients and employees.

MATERIALS AND METHODS

The institution examined has a reputation of being one of the most modern and well equipped for such patients in Japan. As of September, 1986, the number of patients accommodated was 190 (135 boys and 55 girls), aged between 8 and 26 years, and the number of workers was 131. The institution consists of a building of four levels of floors and each floor is set up as a self-contained unit. Boys and girls are accommodated separately; the former on the 2nd and 4th floors and the latter on the 3rd floor. All the patients belong to two classes: one is the life class to establish basic habits for daily life and the other is the training class to evaluate and improve mental ability. In some neighboring rooms, patients with the same degrees of adaptation live together to make a life class (9-20 patients in number). On the other hand, the training class consists of patients with the same interests

and potential, irrespective of sex or placement in the life class. The training classes are located on the 1st floor of this institution. This means that only in the training class patients can make contact with those from other floors.

Stool examinations and the indirect fluorescence antibody test (IFA) were chosen as epidemiologic tools. Modes of transmission were deduced from both parasitological and serological surveys.

Stool examinations on direct smears were made in a conventional manner to detect trophozoites or cysts of *E. histolytica*. To increase its efficiency, a formalin-ether concentration technique (MGL method) was also employed. The species of amebae were identified with iron-hematoxylin-stained specimens, when necessary.

Serodiagnosis (IFA) of amebiasis was performed in the same manner as that for the histochemical detection of anti-*Trypanosoma cruzi* antibody (7), except that FITC-conjugated anti-human IgG was used instead of the peroxidase-labeled antibody. Axenic *E. histolytica* trophozoites, strain HM-1: IMSS and HK-9, were used as the antigens. The parasites were provided by Dr. L. Diamond, NIH, Bethesda, MD, USA and have been maintained in our laboratory for about five years. The amebae were grown in BI-S-33 medium as developed by Diamond et al (8). The amebic antigen was prepared as follows: trophozoites in log phase were harvested by centrifugation and washed with an excess of 10 mM phosphate-buffered saline (PBS), pH 7.4. The amebae were fixed with 3% formalin in PBS for 30 min at 4 C and washed three times in PBS. The fixed amebae were resuspended in PBS and smeared on each glass slide to an amount of 2×10^3 cells. The slides were air-dried at room temperature and stored at -70°C until used. FITC-conjugated goat anti-human IgG was obtained from MBL Co. (Nagoya, Japan) and used at a 50-fold dilution. After a preliminary evaluation of this IFA with pooled positive and negative sera for *E. histolytica*, a titer of 1:64 or greater was judged as positive.

Statistical significance was assessed by Student's *t* test and the χ^2 test with Yates' correction.

RESULTS

Stool examinations demonstrated that none of the workers was positive for *E. histolytica*. Serologically, however, 6.5% of 93 patient-care staffs were judged as positive. Moreover, 10.5% of the remaining 38 office personnel and house-keeping workers, who usually do not have direct contact with the patients, were positive with low IFA titers.

The results of stool examinations and IFA tests of the 190 patients are summarized in Table I. Twenty percent of the patients (38 in number) were

Table I. The results of stool examinations and IFA tests for amebic infection in the patients

		IFA test		Subtotal
		Negative	Positive	
Stool exami- nation	Negative	113 (59.5%)	39 (20.5%)	152 (80.0%)
	Positive	5 (2.6%)	33 (17.4%)	38 (20.0%)
	Subtotal	118 (62.1%)	72 (37.9%)	190 (100.0%)

A titer of 1:64 or higher was judged as IFA-test positive.

positive by stool examinations, while 37.9% (72 patients) were seropositive. Cysts and/or trophozoites of other parasitic protozoa such as *E. coli*, *Endolimax nana* and *Giardia lamblia* were also detected in 14, 8 and 2 cases, respectively, as simple or combined infections with *E. histolytica*. Helminth eggs were not detected by stool examinations. Only four of the 38 stool-positive cases were found to be symptomatic (amebic dysentery). The remaining 34 subjects seemed to be asymptomatic cyst carriers.

The data obtained by IFA tests were matched with the positive and negative cases shown by stool examinations to see if there was any correlation (Table I). Among the stool-negative group, 39 patients (20.5% of the total patients) were seropositive, while 33 of the 38 stool-positive subjects were seropositive (17.4%). One of the seropositive group with a negative stool was diagnosed as having an amebic liver abscess, and one of the seronegative group with a positive stool was found to possess antibodies against *E. histolytica* 3 months later.

The data obtained by stool examinations and IFA tests are summarized by the sex and age of patients in Table II. The prevalence shown by stool examinations seemed independent of gender; seropositivity in boys was 39.9%, in girls 34.6%; the slight difference was not statistically significant. Stool positive boys (mean age 15.6 ± 3.4 years) were significantly younger than the negative

Table II. Incidence of *E.histolytica* by sex and age of the patients

	Number tested (%) mean age (yrs)	Stool examination		Serological test		Both-tests	
		Number negative (%) mean age	Number positive (%) mean age	Number negative (%) mean age	Number positive (%) mean age	Number negative (%) mean age	Number positive (%) mean age
Boys	135 (100)	108 (80.0)	27 (20.0)	82 (60.7)	53 (39.3)	78 (57.8)	23 (17.0)
	17.7 $\pm$ 5.8	19.1 $\pm$ 6.5	15.6 $\pm$ 3.4 ^b	18.6 $\pm$ 6.0	16.2 $\pm$ 5.0 ^b	18.7 $\pm$ 6.2	15.1 $\pm$ 3.5 ^a
Girls	55 (100)	44 (80.0)	11 (20.0)	36 (65.4)	19 (34.6)	35 (63.6)	10 (18.2)
	16.2 $\pm$ 4.7	16.6 $\pm$ 4.8	16.1 $\pm$ 3.8	17.0 $\pm$ 4.9	14.7 $\pm$ 3.7	17.0 $\pm$ 5.0	16.1 $\pm$ 4.1
Total	190 (100)	152 (80.0)	38 (20.0)	118 (62.1)	72 (38.0)	113 (59.5)	33 (17.4)
	17.3 $\pm$ 5.5	17.7 $\pm$ 5.7	15.7 $\pm$ 3.5 ^a	18.1 $\pm$ 5.7	15.8 $\pm$ 4.7 ^a	18.2 $\pm$ 5.8	15.4 $\pm$ 3.7 ^a

a and b: statistically significant against each negative patient, $\underline{p} < 0.05$ and $\underline{p} < 0.02$, respectively.

Table III. Correlation between seropositivity for amebic infection and mean age and length of stay at the institution of the patients

	Total	IFA negative	IFA titer			subtotal
			x 64	x 256	x 1024	
Number	190	118	31	35	6	72
Mean age (years)	17.3 ± 5.5	18.1 ± 5.7	16.5 ± 6.1	15.7 ± 4.0 ^b	13.3 ± 3.4 ^b	15.8 ± 4.7 ^a
Length of stay (years)	5.9 ± 5.1	6.2 ± 5.2	5.9 ± 5.7	5.3 ± 3.9	3.8 ± 3.5	5.4 ± 4.6

a and b: statistically significant against IFA-negative patients, $\underline{p} < 0.01$ and $\underline{p} < 0.05$, respectively.

ones (19.1 ± 6.5 years, $P < 0.02$); seropositive boys (mean age 16.2 ± 5.0 years) were also younger than the negative ones (18.6 ± 6.0 years, $P < 0.02$). Moreover, boys positive in both tests (mean age 15.1 ± 3.5 years) were also younger than the negative ones (18.7 ± 6.2 years, $P < 0.05$). The positive results for boys are reflected in the findings for all the patients. Accordingly, it was shown that amebic infection was concentrated in younger patients.

Such a trend can readily be accepted from the data summarized in Table III. As the IFA titer increases, the age of seropositive patients becomes younger. In other words, the younger patients appeared to be more heavily infected with *E. histolytica* than the older ones. This speculation was supported by the finding that all of the previously-mentioned four symptomatic patients were younger (mean age 11.8 ± 1.0 years, $P < 0.05$) than IFA-negative patients (18.1 ± 5.7 years). Moreover, these four symptomatic patients were in the institution for 1.3 ± 0.8 years. The length of stay was shorter than the average for IFA-negative patients (6.2 ± 5.2 years, $P > 0.1$, not to a significant extent). In general, the shorter the stay of seropositive patients in the institution, the higher the IFA titer. Namely, all these observations indicate that active, symptomatic amebiasis is concentrated in younger patients with a short period of stay in the institution.

The relationship between severity of mental retardation and amebic infection was investigated (Table IV). The patients in the institution were separated by degree of mental retardation into four categories, based on the intelligence quotient (I. Q.) classification system of WHO (9) as follows: mildly retarded (I.Q. 51-75), 6.4%; moderately (I.Q. 36-50), 26.3%; severely (I.Q. 21-35), 50.0%; and profoundly (I.Q. 0-20), 17.4%. Approximately 90% of the cases positive by stool examinations were in the severely and profoundly retarded groups. The incidence of amebic infection in serodiagnosis was also significantly higher in patients with a severe or profound degree of retardation than in the mild or moderate group (62/128 and 10/62, respectively, $\chi_0^2 < 0.001$). In addition, the percentages of heavily retarded patients (severe and profound degrees) in the groups for each titer of IFA were: $\times 64$, 87.1% ($\chi_0^2 < 0.005$); $\times 256$, 85.7% ($\chi_0^2 < 0.005$); and $\times 1,024$, 83.3% (the average for seropositivity was 86.1%. $\chi_0^2 < 0.001$) (Table IV). Accordingly, it is suggested that the heavily retarded patients have a higher risk of amebic infection and are probably the major source of new infections.

Moreover, 75.8% of the heavily retarded patients (I.Q. 0-35) are associated with abnormal idiosyncratic activities such as pica, hyperkinesis with developmental delay, fecal play and onanism. As demonstrated in Table V, it is easily understandable that the patients with idiosyncrasies relating to hand-to-

Table IV. Correlation between the results of stool examinations and IFA tests for amebic infection in the patients and their I. Q. levels

M. D. (I Q)	Number (%)	Stool positive (%)	Number (%) at IFA titer				
			negative	x 64	x 256	x 1024	seropositive (%)
mild (51-75)	12 (6.4)	2 (5.3)	9	1	1	1	3 (4.2)
moderate (36-50)	50 (26.3)	1 (2.6)	44	3	4	0	7 (9.7)
severe (21-35)	95 (50.0)	27 (71.1)	50	22	20	4	46 (63.9)
profound (0-20)	33 (17.4)	8 (21.1)	15	5	10	1	16 (22.2)
Total	190 (100)	38 (100)	118	31	35	6	72 (100)

M. D.; mental deficiency, I. Q.; intelligence quotient.

Table V. Correlation between the results of stool examinations and IFA tests for amebic infection in the patients with behavioral idiosyncrasies

Idiosyncrasies	Number	Number stool positive (%)	Number IFA positive (%)
Pica	27	6 (22.2)	13 (48.2)
Fecal play	16	1 (6.3)	6 (37.5)
Onanism	7	2 (28.6)	3 (42.9)
Pica and Fecal play	28	13 (46.4)	22 (78.6)
Pica and Onanism	6	2 (33.3)	4 (66.7)
Fecal play and Onanism	0	0 (0.0)	0 (0.0)

mouth (pica and fecal play) were more frequently positive by the stool examination and by the serological test. For example, positive results for patients with pica, fecal play or both pica and fecal play by stool examinations were as follows: 6/27 (22.6%), 1/16 (6.3%) and 13/28 (46.4%), respectively; by serological tests, they were 13/27 (48.2%), 6/16 (37.5%) and 22/28 (78.6%), respectively. These values for patients with both idiosyncrasies are statistically significant against subjects with fecal play ($\chi_0^2 < 0.005$). These results suggest that amebic infection in the heavily retarded patients is related to idiosyncrasies such as hand-to-mouth or object-to-mouth (pica and fecal play).

To find out more about the background of this outbreak, the relationship between positive patients in each life class and those in their training class was investigated (Table VI). Over two-third of patients were found to be positive for *E. histolytica* in 2C, 2D and 2E in Life classes, while similarly high rates of infection were observed also in "1", "5" and "7" in Basic training classes, and in "1" in Care and training classes. Eleven positive patients in life class 2E lived in four rooms (** in Fig. 1). Moreover, the positive patients in Basic Training class "5" were housed in rooms on the 2nd and 3rd floors (* in Fig. 1). The positive cases in life classes tended to concentrate in some training classes.

The patients who were positive by stool examinations and/or IFA tests were treated with metronidazole, 1,000 mg, tid, for 10 days, immediately after the

Table VI. Number of patients positive for *E. histolytica* separated by training and life classes

Life class	Training class																				Sub- Total
	Basic training							Care and training				Rehabilitation training									
	1	2	3	4	5	6	7	1	2	3	4	1	2	3	4	5	6	7	8	9	
2A	0/1*	1/1	0/2		3/0				0/1												4/5
2B		0/4	1/4																		1/8
2C	2/0		1/0	2/1	4/0																9/1
2D			1/0	2/3	2/0	3/1			1/0												9/4
2E							5/0	3/0		3/0											11/0
2F						4/2	1/0			0/2		3/1	0/2								8/7
3A	1/0						0/1					3/2	1/2								5/5
3B	2/1	2/1	0/1	0/1	1/0																5/4
3C		1/0		1/0	1/0		2/1	0/1	1/1												6/3
3D						0/2						1/4	1/4	1/2							3/12
3E															0/3	1/1	0/5	0/2			1/11
4A									0/1	2/7											2/8
4B							1/0			0/1	0/5	1/2	0/6								2/14
4C							0/2	1/0		1/0	2/2		0/1	0/1							4/6
4D															1/2	3/4	0/2	0/2			4/10
4E															1/1	0/1	0/5	2/4	0/2	0/2	3/15
Subtotal	5/2	4/6	3/7	5/5	11/0	7/5	9/4	4/1	2/3	3/7	5/5	4/11	6/10	1/11	2/6	4/6	0/12	2/8	0/2	0/2	77/113

*: number of positive patients / number of patients negative for E. histolytica

Fig. 1. Distribution of patients positive in the stool examination and/or IFA test for *E. histolytica* in each life class.

- : Patients negative for *E. histolytica*
- ◐: Asymptomatic patients positive in stool examination (seronegative cyst carriers)
- ◑: Asymptomatic patients positive in IFA test
- : Asymptomatic patients positive in both tests (seropositive cyst carriers)
- ◼: Symptomatic patients positive in IFA tests (amebic liver abscess)
- ◼: Symptomatic patients positive in both tests (amebic dysentery)
- * and **: see text.

survey. The efficacy of treatment was evaluated monthly by stool examinations and trimonthly by IFA tests. After the treatment, 75% of the stool-positive patients became negative for cysts of *E. histolytica*. Up to April, 1987, several stool examinations uncovered seven new asymptomatic carriers, although mass treatment with the amebacidal regimen for 5 days was conducted for all the negative patients. Treatment with the same drug plus antibiotics (tetracycline or chloramphenicol) for 10 days was repeated for the 12 recurrences and the seven new carriers. Periodic check-ups have been performed and, as of August 1988, new or recurrent carriers were not found.

DISCUSSION

The majority of institutionalized individuals found to harbor *E. histolytica* have been reported to be asymptomatic in the Western countries, and the fact that amebic dysentery is seen commonly and deaths from invasive amebiasis have also been reported from four institutions in the United States (4). To understand the status of amebic infection in mental institutions, further demographic analysis is necessary.

Transmission of enteric protozoa is related to contaminated food or water. However, the present amebic infection was not correlated with food or water. The main water supply was derived from the town water department and was chlorinated. Although the kitchen in this institution provides food for all resident patients and staff members, none of the employees was positive by stool examinations. There is little doubt that this outbreak was due originally to domestic amebiasis, since few, if any, patients or staff members have been abroad. The high incidence of seropositivity in the stool-positive group (33/38; 86.8%) may be due to infection with a pathogenic strain of *E. histolytica*. Indeed, the result of zymodeme study supposed that the amebae responsible for this outbreak originated from a single pathogenic strain (details will be described elsewhere).

The epidemiological analysis of mode of transmission was very difficult. In this amebic outbreak, patients suffering from invasive amebiasis and patients positive by both tests (stool examinations and IFA tests) were clustered in some life or training classes. These results suggest that *E. histolytica* was passed by person-to-person contact. Because patients from one floor can make contact with those from other floors only in a training class, it appears reasonable to believe that transmission occurred in the training classes. Moreover, the increased risk of

amebic infection among the heavily retarded patients in this institution seems to have resulted from environmental contamination with amebic cysts, and from hand-to-mouth or object-to-mouth contact.

In general, the incidence of amebic infection is related to the sex and age of the host. Males have been found to be infected more frequently with this parasite than females (11) and the highest incidence has been found among persons at ages between 26 and 30 (11), and also among those at ages between 46 and 56 (12). According to the Statistics of Communicable Diseases issued by the Japanese Government, the highest incidence of amebiasis was observed in males between 35 and 59 years of age (13). On the other hand, the present outbreak of amebiasis revealed a sex-independent but age-dependent distribution. In contrast to the previous report by Sexton et al. (5), the amebic infection in the present research primarily occurred in the young group of heavily retarded patients (I. Q. 0-35). Similarly, the amebiasis outbreak in a kibbutz population due to infant-mother transmission of amebae showed two peaks in incidence, namely among persons of 0 to 9 years and 30 to 49 years (14). This age distribution seemed to be compatible with those found in other reports. These data suggest that amebic infection is generally more widespread among younger retarded individuals.

The present outbreak of amebiasis in the institution revealed some important problems. According to the diary of the institution, the first case of dysentery is believed to have appeared in 1980 on the 4th floor, with subsequent cases appearing on the 2nd floor in 1982 and on the 3rd floor in 1985. So this disease may have been present in this institution, one of the best-equipped facilities for the mentally retarded in Japan, for at least the past 6 years. It took a considerable period of time to recognize the prevalence of amebic infection inside the institution. The oversight of the prevalence of the protozoan disease in the present institution may be due to the widespread belief in the early 1980s that amebiasis in Japan was on the decline.

At present, the number of mentally retarded patients in Japan is over 66,000, and approximately half of them are housed in 321 facilities (15). The fact that the mentally retarded patients exhibit abnormal idiosyncratic behavior is well known. In these facilities, therefore, outbreaks of various communicable diseases have frequently been observed. Though the majority of cases of amebiasis in institutions for mentally retarded is asymptomatic, a significantly higher rate of seropositivity than that in other psychiatric groups (5) and that of the deaths caused by the disease have been reported (4). The potential for invasive amebiasis is now being emphasized. Accordingly, constant health checks of institutionalized

populations should be carried out by supervisors of these facilities and by public health officers of each local government.

ACKNOWLEDGEMENTS

We wish to thank Ms. K. Kurihara and Ms. Y. Kato of the Department of Parasitology, Tokai University, for excellent technical assistance.

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