

Original

A Pilot Study for the Detection of Protozoa Infections of the Gut at Autopsy

Masafumi ABE^{1,7)}, Hiroyuki FUKUTOMI³⁾, Tetsuro SHIRAKURA¹⁾,
Wei-Hua ZHAO¹⁾, Hiroko ASAH⁴⁾, Miki KUSHIMA⁶⁾,
Akira SHIOKAWA⁵⁾, Kenji YAGITA⁴⁾, Kazuo TANAKA¹⁾,
and Satoshi KIMURA²⁾

Abstract : In order to investigate the incidence of latent infections by parasites within the human digestive tract, we examined fresh stool samples from the colon of 31 patients (37–90 years, median age 71 ; 26 men and 5 women) collected within 12 hours of death. These subjects had been admitted to a university hospital in Yokohama, Japan, and died between April 2007 and December 2008 from causes other than parasitosis. Stool samples were fixed and stained for microscopic analysis, and PCR analysis for *Entamoeba histolytica* was performed for the parasite-positive samples. Results showed that ten out of 31 subjects were infected by *E. histolytica* only, one subject was infected by *Giardia intestinalis* only, and four subjects were infected by both *E. histolytica* and *G. intestinalis*. These findings are in contrast to conventional theories concerning general parasite infection in Japan, and indicate continuous or latent infection within the human digestive tract. The presence of pathogens such as *E. histolytica* and *G. intestinalis* in elderly or immuno-compromised patients is a serious issue and warrants further attention as a public health issue, particularly in relation to its mode of transmission.

Key words : post-mortem investigation, pathogenic protozoa infection, *Entamoeba histolytica*, *Giardia intestinalis*

Introduction

It has often been reported that the dissection of human corpses identifies the presence of various parasites and parasitic diseases^{1,2)}. However, there are few cases which epidemiologically investigate the kinds of parasites in pathological subjects³⁾ within the general spectrum. As such, we conducted a parasitological investigation on human subjects shortly

¹⁾ Department of Microbiology, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan.

²⁾ BML Inc, and Central Clinical Laboratory, Showa University Northern Yokohama Hospital.

³⁾ FTB Science Co. Ltd.

⁴⁾ Department of Parasitology, National Institute of Infectious Diseases.

⁵⁾ Department of Pathology, Showa University Northern Yokohama Hospital.

⁶⁾ Division of Diagnostic Pathology, Showa University Hospital.

⁷⁾ Department of Biomedical Technology, Faculty of Biomedical Engineering, Toin University of Yokohama.

after death in order to examine latent infections of human intestinal parasites.

Materials and Methods

Study design

We investigated the species of parasites in stool samples from the colon of patients obtained within 12 hours of death. These subjects had been admitted to university hospital in the Kanto district in Japan, and the main cause of death had been diagnosed as other than parasite diseases. The period of investigation was 1 year and 9 months, from April 2007 to December 2008. The total number of subjects examined was 31 (the number of pathologic subjects dissected at the hospital is 30–40 per year). Signed consent to take samples for parasitological examination was obtained from the patients' family, and each sample was properly obtained under a pathologic physician's instruction and under the permission of the commission of the university (admission No. 708).

Parasitological diagnosis

Each fresh stool sample was prepared for microscopic examination using the direct staining method, formalin-ether method, sucrose floatation technique using 10% formalin, Kohne staining and MIF solution fixation. We also used enzyme linked immunosorbent assay (ELISA), [Wampole, U.S.A., Catalog No.30404; U.S.Patent #5,272,058 (2006)] for the detection of *Entamoeba histolytica*, which has recently been noted to have an increased incidence in Japan, and examined the presence of the associated DNA using polymerase chain reaction (PCR)⁴⁾.

Results

Summary of pathological examination (Tables 1 and 2)

Table 1 shows general information, major cause of death and other information before death for each subject. The total number of subjects examined for the period April 2007 to December 2008 was 31 (Table 1-1; 1-2), and comprised 26 males and 5 females, aged from 37 to 90 years of age, with a median age of 71. Major causes of death were cancer (12 subjects), heart disease (6), respiratory disease (5), liver disease (5) and brain disease (3). General observation of the digestive tracts was normal in 28 subjects, and three had been identified with an ulcer or polyp. Symptoms before death were normal in 17 patients, 3 had diarrhea, 2 had bloody bowel discharge, 1 had an occult positive blood test, and eight were not described. All subjects positive for parasites had a poor nutritional condition when they were admitted to the hospital, i.e. total serum protein was < 6.5 g/mL, which is the normal standard. This included cases which had received anti-cancer drugs or antibiotics, indicating they were at a very low immunological condition at the time of death. There were no cases in which examination for parasites had been performed when digestive symptoms were apparent before death.

Table 1-1 ; 1-2. General information and major cause of death for each subject^a

Table 1-1.

Sample No.	Detected parasites	Age	Sex	Major cause of death	Health condition before death
1	negative	90	male	congestive heart failure	low immunological condition, bronchitis, occult blood stool
2*	<i>E. histolytica</i>	72	male	sepsis & pneumonia	ulcer of the esophagus cancer of the large intestine
3	negative	75	male	cerebral infarction led to dysphasia	low immunological condition, polyp of the digestive tract
4	negative	72	male	heart failure	dilated cardiomyopathy
5	negative	64	male	aspiration pneumonia & septic shock	inflammation of the duodenum, polyp of the ascending colon
6*	<i>E. histolytica</i>	87	male	respiratory failure from hemothorax	low immunotogical condition, heart hypertrophy, the normal digestive tract
7	negetive	61	female	recurrence & metastasis of the breast cancer	low immunological condition, the normal digestive tract
8*	<i>E. histolytica</i>	82	male	rupture of the large artery	low immunological condition, the normal digestive tract
9*	<i>E. histolytica</i> & <i>G. intestinalis</i>	83	male	malignant melanoma of the esophagus	low immunological condition, the normal digestive tract
10*	<i>G. intestinalis</i>	71	male	osmotic cerebral disease	low immunological condition, tube feeding, crea emacation
11	negative	68	male	heart failure by arrhythmia	good immunologicat condition
12	negative	74	male	frequent metastasis of cancer of the bladder	low immunologiceal condition, administration of anticancer drags
13*	<i>E. histolytica</i> & <i>G. intestinalis</i>	63	male	cancer of the stomach & pneumonia	low immunological condition, administration of anticancer drags
14*	<i>E. histolytica</i>	57	male	acute hepatitis by drugs or drinking	low immunological condition
15	negative	48	male	mitochondrial encephalopathy	low immunological condition
16*	<i>E. histolytica</i>	80	female	cancer of the liver	pain of abdomen, diarrhea, vomit, administration of antibiotics
17	negative	82	male	thymoma	low immunological condition, no description of the digestive symptom
18*	<i>E. histolytica</i> & <i>G. intestinalis</i>	65	male	frequent metastasis of cancer of the colon	retention of the ascites, unknown cause of bloody bowel discharge
19	negative	75	male	small cell carcinoma of the lung	no description of the digestive symptom
20*	<i>E. histolytica</i>	75	male	cirrhosis & hepatocellular carcinoma	much ascites, insufficiency of the liver, no description of the digestive symptom
21	negative	74	female	acute myocardial infarction	low immunological condition, no description of the digestive symptom
22	negative	78	female	pneumonia	low immunological condition, vomiting, no bloody bowell discharge
23*	<i>E. histolytica</i>	71	male	insufficiency of the liver	strong swelling of the abdomen, carcinoma in the liver, tarry stool
24	negative	69	male	cancer of the esophagus	low immunologicsl condition, no description of the digestive symptom
25*	<i>E. histolytica</i>	62	male	cancer of the lung & esophagus, the pleomorphic carcinoma	strong abdominal swelling & pain by the metastasis to the abdomen

Table 1-2.

Sample No.	Detected parasites	Age	Sex	Major cause of death	Health condition before death
26*	<i>E. histolytica</i> & <i>G. intestinalis</i>	79	male	chronic obstructive pulmonary disease	stool occult blood, essential anemia, liquid diarrhea
27	<i>E. histolytica</i>	65	male	cancer of the lung	low immunological condition, no description of the digestive symptom
28	negative	67	male	hematemesis by alcoholic cirrhosis	acute insufficiency of the kidney, storage of the abdominal inflammation
29	negative	78	male	chronic obstructive pulmonary disease, pneumothorax	diarrhea by MRSA enteritis
30	<i>E. histolytica</i> & <i>G. intestinalis</i>	77	male	arrhythmia, sudden death	low immunological condition, bedridden by cerebral infarction & diabetes
31	negative	37	female	alcoholic cirrhosis & cerebral hemorrhage	bloody bowel discharge by cirrhosis

* : a parasite positive case. All examined subjects were Japanese who were residents of Japan, had not travelled overseas and did not have AIDS.

Results of parasitological examination (Table 2)

As shown in Table 2 (2-1 ; 2-2), 15 cases out of 31 were positive for pathogenic parasites, including ten cases of *E. histolytica*, one case of *G. intestinalis*, and four cases of both *E. histolytica* and *G. intestinalis* infection. This result indicates a high infection rate (48%, 15/31), with *E. histolytica* being the predominant pathogenic parasite (93%, 14/15). Examination of the stool samples indicated seven cases of normal stools, 12 cases of loose stools, seven muddy stools, four diarrhea stools and one case of a mucous bloody stool. Within the parasite positive subjects, there were three cases of normal stools, seven cases of loose stools, two cases of muddy stools and one case of bloody mucous stool, suggesting that some symptoms related to the digestive tract had been occurring in the parasite positive subjects.

Confirmation of E. histolytica infection by PCR reaction (Table 2, Fig. 1 & 2)

As shown in Table 2 (2-1 ; 2-2), Fig. 1 & Fig. 2 for the *E. histolytica* positive samples, the results of the microscopy were in accordance with those of the antigen examination, suggesting that this parasite was established inside the colon of the subjects, although complete conformity was not obtained between the antigen detection and the PCR reaction for unknown reasons. For the *G. intestinalis* infection, four out of five cases (80%) were found with co-infection of *E. histolytica* in the parasite positive subjects.

Table 2-1 ; 2-2. Results of parasitological examination^a

Table 2-1.

Sample No.	State of stool sample	Microscopy	Antigen exam. for <i>E. histolytica</i>	PCR reaction
1	normal	negative	negative	nd
2*	diarrhea	<i>E. histolytica</i>	positive	positive
3	normal	negative	negative	nd
4	normal	negative	negative	nd
5	normal	negative	negative	nd
6	normal	<i>E. histolytica</i>	positive	negative
7	normal	negative	negative	nd
8*	normal	<i>E. histolytica</i>	positive	positive
9*	mucous bloody	<i>E. histolytica</i> & <i>Giardia intestinalis</i>	positive	negative
10*	loose stool	<i>G. intestinalis</i>	negative	negative
11	loose stool with green brown color	negative	negative	nd
12	muddy stool	negative	negative	nd
13*	loose stool	<i>E. histolytica</i>	positive	positive
14	loose stool	<i>E. histolytica</i>	positive	positive
15	loose stool	negative	negative	nd

Table 2-2

Sample No.	State of stool sample	Microscopy	Antigen examination for <i>E. histolytica</i>	PCR reaction for <i>E. histolytica</i>
16*	black, loose stool	<i>E. histolytica</i>	positive	positive
17	brown, muddy stool	negative	negative	nd
18*	brown, muddy stool with a bad smell	<i>E. histolytica</i> & <i>G. intestinalis</i>	positive	positive
19	brown, muddy stool	negative	negative	nd
20*	green, solid stool	<i>E. histolytica</i>	positive	positive
21	brown, solid stool with a bad smell	negative	negative	nd
22	brown, diarrhea	negative	negative	nd
23*	green & brown diarrhea	<i>E. histolytica</i>	positive	positive
24	brown, loose stool	negative	negative	nd
25*	brown, loose stool	<i>E. histolytica</i>	positive	positive
26*	loose stool	<i>E. histolytica</i> & <i>G. intestinalis</i>	positive	positive
27*	brown, muddy stool	<i>E. histolytica</i>	positive	positive
28	brown, loose stool	negative	negative	nd
29	brown, diarrhea	negative	negative	nd
30*	brown, loose stool	<i>E. histolytica</i> & <i>G. intestinalis</i>	positive	negative
31	brown, loose stool	negative	negative	nd

^a* : a parasite positive case; nd : not determined because of antigen negative sample for pathogenic parasites ; positive : positive for the pathogenic protozoa; negative : negative for the pathogenic protozoa.

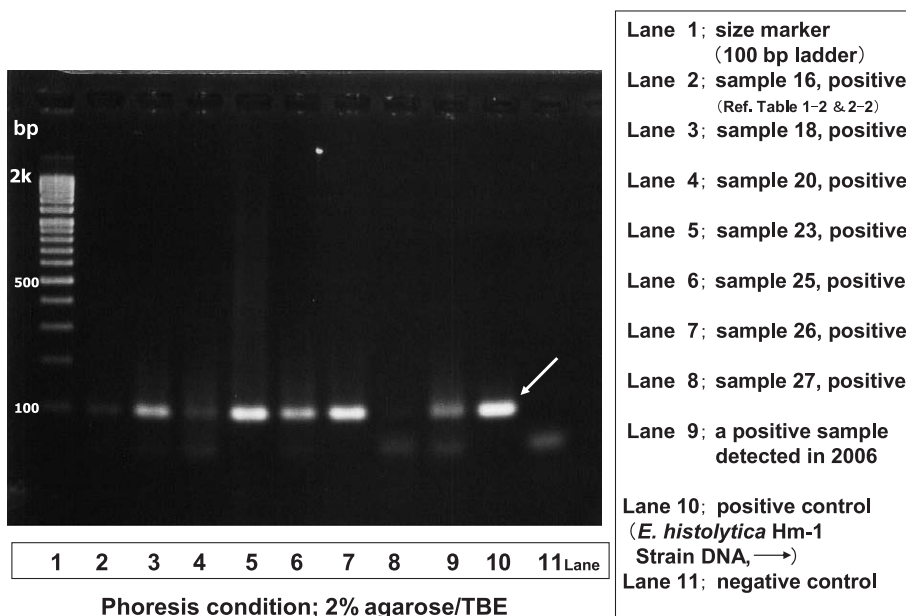
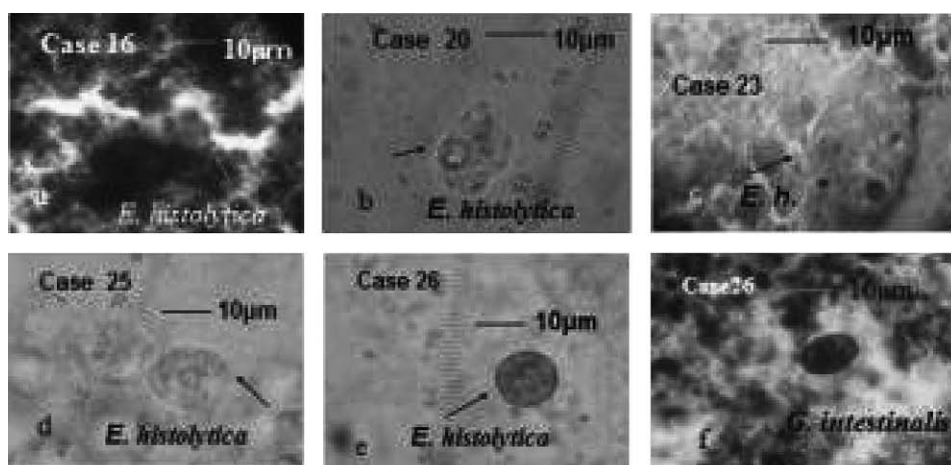


Fig. 1. PCR reaction of electrophoresis from *E. histolytica* antigen positive samples^a

^aThis photograph was taken by Dr. Kenji Yagita. Lanes 2, 4 and 8 show a thin band at 100 bp which corresponded to that of the positive control (lane 10), and can therefore be considered positive for *E. histolytica*. Subject 15 was positive for the antigen, however, was negative for the PCR reaction.



● Case No. in each figure was in accordance with the same No. of each sample listed in Table 1 or 2.

Fig. 2. Parasites detected in the microscopic investigation

a: *E. histolytica* (trophozoite), ×1000, Kohne staining (sample 16); b: *E. histolytica* (trophozoite), ×1000, sucrose-floatation technique (sample 20); c: *E. histolytica* (trophozoite), ×1000, sucrose-floatation technique (sample 23); d: *E. histolytica* (trophozoite), ×1000, MIF fixation method (sample 25); e: *E. histolytica* (cyst), ×1000, Formalin ether method (sample 26; f: *G. intestinalis* (cyst), ×1000, Kohne staining (sample 26).

Discussion

It has been well documented that pathological dissections of subjects reveal various parasites and confirm the existence of new parasitic diseases¹⁾. Parasite detection methods other than dissection and stool examination from patients have also been well reported²⁾. However, there have been few epidemiological studies which focus on the parasite species of general post-mortem pathological subjects³⁾.

We therefore conducted a parasitological study of pathological subjects just after death to confirm latent infection of parasites within the human digestive tract. As shown in Table 1 (1-1; 1-2), the main causes of death among the subjects were various but the cases in which parasites of the digestive tract were mainly responsible were not found. However, parasitological investigation of the stools within the colons just after death showed a high percentage of samples (15 out of 31 samples in total) were infected with *Entamoeba histolytica* or *Giardia intestinalis*. The rate of infection was much higher than the infection rates detected among general Japanese residents for general parasites since 1995 (below 0.1%)⁵⁾, indicating that this is not only a pathological or a parasitological problem but also an important public health problem in our society. For *Giardia* infection, many latent infective cases have been reported to date, including those of a study conducted at a general complete medical check up which indicated high percentages of infection (ca 0.3%)⁵⁾. Furthermore, for amebic infectious diseases, a large proportion (ca 90%) of the estimated infected people (ca 5 billion in the world) were reported to be asymptomatic^{1,2)}. However, our findings detected amoeba, *E. histolytica*, a pathogenic protozoa, indicating that this parasite was important as a latent source of infection, with the risk of getting worse in each fundamental disease or immunological condition indicated as an opportunistic pathogen of each subject.

The precise mechanism of parasite infection and its establishment in each subject is not clear at present. However, it is suspected that during the process of death, an immunosuppressive condition was produced by the advanced age of the patient, the chronic malnutrition at the time of hospitalization, by the use of anti-cancer drugs, or by long-term administration of antibiotics which eliminated microbes other than the protozoa, and may have led to suitable conditions within the digestive tract for parasites. Furthermore, referring to a report⁶⁾ by NIH in Japan which summarized the recent incidence of amebiasis, the subjects of the current study (median 71 years of age) were mainly at a young and active age in and around the period of World War II, when they may have had increased exposure to parasites in their daily lives. In the current study, all subjects were Japanese, residents of Japan and had not travelled overseas. It should be noted, however, as in other reports^{6,7)} that infection from STD^{7,8)}, daily food (rice or fresh vegetables)⁹⁾ at a younger age and its long sustainable infection¹⁰⁻¹²⁾ or latent infection¹³⁻¹⁵⁾ are potential causes of amoeba infection in our case report. Moreover, the high rate of detection of *E. histolytica* in this study requires more thorough investigation. In previous studies, asymptomatic cyst carriers of *E.*

histolytica, but not *E. dispar*, were detected with a high rate of infection (1.8 ~ 24.7%) in the residents of institutions for the mentally retarded in Kanagawa prefecture^{4,16)} or in Yamagata prefecture (48.7%)¹⁷⁾ in Japan. High infection rates among Western homosexual men (20 ~ 30%)¹⁾ and Japanese homosexual men in the big cities of Japan (Tokyo, Yokohama, Osaka) were also reported, and associated with the incidence of AIDS⁷⁾.

For our study, we continued our investigation until the end of December 2009, with a total of 100 subjects from other hospitals in our university (unpublished data of M. Abe). The rate of *E. histolytica* detected among those samples was also high (41/100), but definitive clinical symptom cases positive to this protozoa were few, indicating the possibility that the protozoa exists at the molecular level but remains in an asymptomatic state, although more daily attention for opportunistic infection should be given for these patients. Previous reports^{4,18)}, suggest that in asymptomatic cases, cyst carriers were predominant, especially in institutions for the mentally retarded in Japan, and the diversity of the genotype of *E. histolytica* was observed between the male homosexual population and mentally handicapped persons in institutions¹⁸⁾. In the current study, such an asymptomatic genotype of *E. histolytica* may also be present, although more conclusive analysis was not possible due to the difficulty of culturing cysts from clinical isolates using animal models.

Acknowledgements

We would like to thank Dr. Fuminori Nakamura, Associate Professor of the Microbiology Department, School of Medicine, Showa University, and Dr. Tetsuo Kodaka, Associate Professor of Department of Oral Anatomy and Developmental Biology, Showa University, School of Dentistry, for reading and correcting our article. A part of this investigation was presented in a poster session of the XIIth International Congress of Parasitology (ICOPAXII, 2010, Melbourne)¹⁹⁾.

References

- 1) Yoshida Y and Arizono N: Illustrated Human Parasitology 7th ed., Nanzando, Tokyo, (2006) (in Japanese)
- 2) Introduction. In: Encyclopedic Reference of Parasitology: diseases • treatment • therapy, 2nd ed., Heinz M (Ed.) Springer, Berlin, pp XVII-XXI (2000)
- 3) Masuda H, Nitta M, Nakamura N, Shiokawa K, Kumura H, Kanda M, Hatano T, Nozu T and Hatta Y: *Cysticercus cellulosae hominis* in Japan - an autopsy case considered to be infested more than 30 years ago and a study of literatures (collecting 354 cases). *J Showa Med Assoc* **40**: 669-688 (1980) (in Japanese)
- 4) Tachibana H, Kobayashi S, Nagakura K, Kaneda Y and Takeuchi T: Asymptomatic cyst passers of *Entamoeba histolytica* but not *Entamoeba dispar* in institutions for the mentally retarded in Japan. *Parasitol Int* **49**: 31-35 (2000)
- 5) Minoru S: Again increasing helminthic diseases. *J Ther* **80**: 136-137 (1998) (in Japanese)
- 6) Ohnishi K: Amebiasis in Japan, 2003-2006. *Infect Agent Surveill Report* **28**: 103-104 (2007) (in Japanese)
- 7) Ohnishi K, Kato Y, Imamura A, Fukuyama M, Tsunoda T, Sakaue Y, Sakamoto M and Sagara H: Present characteristics of symptomatic *Entamoeba histolytica* infection in the big cities of Japan. *Epidemiol Infect* **132**: 57-60 (2004)
- 8) Ohtomo K, Wang S, Masunaga A, Iwamoto A and Sugawara I: Secondary infections of AIDS autopsy cases in Japan with special emphasis on *Mycobacterium avium-intracellulare* complex infection. *Tohoku J Exp Med* **192**: 99-109 (2000)

- 9) Shinoda T, Kimura S, Seki T, Nakamura M, Ikeda H, Murakami K, Kurosawa K, Abe M and Nakamura F : Three female cases of amebic dysentery without history of overseas travel. *Clin Parasitol* **18** : 31-34 (2008) (in Japanese)
- 10) Suzuki Y, Adachi Y, Yasumizu R, Okamura A, Kimura H, Hachimine D, Tienan J, Minamino K, Iwasaki M and Ikehara S : Amebiasis : two autopsy cases where diagnosis could not be established during the life time of the patients. *Jpn J Diagn Pathol* **22** : 25-28 (2005) (in Japanese)
- 11) Kodera T, Takenaka A, Hosoya Y, Matsui J, Maeda S, Kanamori T, Tanaka Y and Nishimura N : One case of amebic colitis which have been passed 5 years chronically in a patient. *Shiga Med J* **28** : 92-93 (2006) (in Japanese)
- 12) Kuwata G, Yoshiike M, Arakawa T, Tu Y, Koizumi K, Kanzawa T, Nakajima H, Monma K, Egawa N, Nemoto T and Funada N : One case of amebic colitis which was diagnosed as the ulceric colitis 30 years before. *Prog Dig Endosc* **68** : 87 (2005) (in Japanese)
- 13) Yamada M, Arizono N and Tachibana H : 3 asymptomatic cyst passers of single *Entamoeba histolytica* and/or mixed infection of *E. histolytica* and *E. dispar* suspected by antibody test and PCR method. *Clin Parasitol* **14** : 34-36 (2004) (in Japanese)
- 14) Murakami H, Murata Y, Awazu Y, Maeda M, Koumoto Y, Izumi K, Koyama M, Tsuchikame N, Ogata I, Negishi T, Kusunoki S, Kako H, Honda Y and Inoyama K : One case of amebic colitis detected at a health check up. *J Gastroenterol Cancer Screen* **44** : 661-662 (2006) (in Japanese)
- 15) Yamanaka Y, Yamano Y, Imai Y, Maeda S, Nakazato M and Matsushita H : One case of asymptomatic amebic colitis diagnosed at the opportunity of a health check up. *Gastroenterol Endosc* **45** : 1793 (2003) (in Japanese)
- 16) Nagakura K : Mass Outbreak of Entamoeba disease. *Infection* **22** : 97-104 (1992) (in Japanese)
- 17) Nishise S, Fujishima T, Ohtani K, Kobayashi S and Nishise Y : The actual state of mass outbreak of *Entamoeba histolytica* in a mentally retarded institution in Yamagata Prefecture in Japan. *J Jpn Assoc Infect Dis* **77** : 922-923 (2003) (in Japanese)
- 18) Nozaki T, Kobayashi S, Takeuchi T and Haghighi A : Diversity of clinical isolates of *Entamoeba histolytica* in Japan. *Arch Med Res* **37** : 277-279 (2006)
- 19) Abe M, Kimura S, Fukutomi H, Asahi H, Yagita K, Shirakura T, Zhao W, Seki T, Nakamura M, Shiokawa A and Tanaka K : High rate existence of pathogenic protozoa latent infection in long term hospitalized patients, a pilot study at autopsy. The XIIth International Congress of Parasitology (ICOPAXII), Melbourne, Australia, 2010 (abstr 706)

[Received September 5, 2011 : Accepted December 15, 2011]