REVIEW ARTICLE

The Diversity of Clinical Isolates of *Entamoeba histolytica* in Japan

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In Japan, amebiasis is domestically transmitted by two major populations: male homosexuals and mentally handicapped persons, which is remarkably different from most other developed countries where *Entamoeba dispar* infection is predominantly observed. Here we briefly summarize epidemiology of amebiasis in Japan. We also review our current understanding of the diversity of *Entamoeba histolytica* clinical isolates in Japan, based on polymorphic genetic markers, clinical representations, and *in vivo* virulence, using an animal model. © 2005 IMSS. Published by Elsevier Inc.

Peculiarities of Amebiasis Endemic in Japan

The most unusual characteristic of amebiasis in Japan is that imported cases comprise only a minor proportion of all cases discovered in the country and cases attributable to domestic transmission dominate (1). We have two major populations infected by amebiasis: male homosexuals and mentally handicapped persons in institutions. We have approximately 500–600 cases of amebiasis reported to the Ministry of Health, Labour and Welfare, including three to four deaths annually. Approximately 90% of the reported cases are male. About 80% of cases have neither a history of traveling in endemic countries nor are they mentally handicapped. Thus, most of the reported cases are likely male homosexuals or bisexuals. Several groups previously reported a very high incidence of amebiasis among male homosexuals based on stool examination and serological tests (2–5). We recently observed sporadic cases of amebiasis transmitted through heterosexual intercourse (1) with an example of female commercial sex workers. Mentally handicapped persons are also severely affected by the disease (6–9). Mass infections of institutionalized mentally handicapped persons were often discovered during an onset of outbreaks as previously reported (6–9).

Recent Survey of Amebiasis in Institutions in Japan

Our recent survey to examine 484 individuals from six institutions [Institute B, C, D (10), E (11), and two other institutes (unpublished)] by a combination of microscopy, antigen capture ELISA, PCR, and serological tests showed that institutions were severely affected by amebiasis. Microscopic demonstration and antigen capture ELISA showed 9.7 and 12.3% overall positive, respectively. Serological tests, e.g., gel diffusion precipitin test and ELISA using whole parasite lysate as antigen showed 4.8 and 31.2% seropositive (unpublished). The positive rates of stool examination and serology varied significantly among institutions, suggesting that the intensity of infection varies among institutions or parasite strains spreading in each institution and vary in virulent competence (see below). Importantly, these cases are often unreported or under-reported for several social reasons. In fact, the cases reported to the Ministry of Health, Labour, and Welfare included no cases of mentally handicapped persons. Thus, the number of amebiasis cases in Japan is largely underestimated. A very high incidence of *E. histolytica* infection in male homosexuals and mentally handicapped persons has not been reported in other developed countries (12–15) except for sporadic cases (16,17) and may be unique to Japan.
Molecular Basis of the Diversity of Entamoeba histolytica Isolates in Japan

Genetic diversity among approximately 40 clinical strains isolated from either male homosexuals or mentally handicapped persons in Japan was determined by high-resolution genotyping based on nucleotide sequencing of two protein-coding (SREHP and chitinase) and non-coding regions (locus 1–2 and 5–6) (10,11). Remarkable differences existed in the degree of heterogeneity of genotypes between the two groups. The strains isolated from male homosexuals are extremely heterogeneous; all the isolates derived from male homosexuals showed distinct genotypes. In contrast, isotypes from mentally handicapped persons were less heterogeneous. Isolates obtained from an institute showed an identical genotype. Moreover, one genotype was detected at three institutions at different times (see below). Because the intensity of infection is low prior to mass infection, probably due to previous mass treatment, mass infection was likely caused by a single source. One genotype was isolated from three institutions [Institutions B, A (10) and E (11)] located in three prefectures (Kanagawa, Shizuoka, and Yamagata) in 1994, 2001, and 2002, respectively. The emergence of the same genotype coincided with the movement of a single infected individual. This case is a good example to demonstrate that molecular fingerprinting is indeed a very reliable tool to determine a source of infection and a route of transmission. In addition, this case also raised serious concern on the effective treatment of amebiasis. The genotypes of Japanese isolates were distinct from four representative reference strains used worldwide (HM1:IMSS c6, SAW755, SAW1627, and SAW1453) (10). In addition, none of 34 isolates from Thailand, Bangladesh, Cambodia, and Indonesia showed genotypes identical to Japanese isolates (11). Thus, the origin of Japanese strains is not understood and should be investigated in future studies.

Diversity of Clinical Manifestations and In Vivo Virulence among Japanese Strains

From a clinical point of view, heterogeneity of virulence attributable to genetic polymorphisms of the parasite likely exists. For instance, when we compared parasitological and serological results between Institutions D and E, which showed a similar rate of infection, notable differences in the serological marker for tissue invasion were observed. While parasitological stool examination gave a similar level of positive rate (28–30% positive) in these institutions, the seropositivity evaluated by gel diffusion precipitin test significantly differed (0 or 16% in Institute D or E, respectively). The mean value of ELISA titer of the infected individuals was also significantly different between the two institutions (optical density at 405 nm of 0.13 or 0.50, respectively). The premise that this is not due to different rates of infection was also supported by the fact that the positive serology rate by ELISA was comparable between the two institutions (54–67%). These data strongly argue for the presence of genetic polymorphisms leading to distinct clinical manifestations.

Experimental animal infection using five isolates categorized into three representative genotypes from mentally handicapped persons also supported this premise. Hamsters were challenged with a direct inoculation of $5 \times 10^3$ trophozoites of CU13 (Institution A), KU19 (B), KU26 (C), KU27 (D), and KU33 (E), cultivated monoxenically with Crithidia fasciculata (18) to the liver, and abscess formation was evaluated a week later. All strains except for KU27 developed liver abscesses, while KU27 failed to cause abscesses even in repeated attempts using a 4-times higher number of amebas (unpublished). These data agreed with the clinical manifestations in the patients infected with these strains and were consistent with the premise that a spectrum of virulence exists among the strains. In vitro virulence is conveniently assessed with the parasite’s capacity to destroy a monolayer of mammalian cells (19). KU27 was incapable of destroying the monolayer of HeLa and Chinese hamster ovary cells, similar to E. dispar trophozoites (unpublished).

These avirulent phenotypes of KU27 are associated with a specific genotype of locus 1–2 type C and SREHP type A, neither of which was found among isolates examined in our laboratory, except two other isolates from the same institution (KU28 and KU29). Whether or not this specific marker is associated with the avirulent phenotype is not known. There is also no causal connection between this particular SREHP type and a lack of virulence. There are no notable differences in clinical manifestations of amebiasis between Japan and other countries. Hepatic, pulmonary, and brain abscesses are seen 5–20% of cases (almost exclusively in male). None of the four genetic markers was found to be associated with a tissue tropism.

References


