# Investigation of an Outbreak of Amoebiasis in a Rehabilitation Institution for Mental Patients in Taichung City

Min-Tsung Lin<sup>1</sup>, Sung-His Wei<sup>2</sup>, Shao-Hui Tsai<sup>1</sup> Yun-Hsien Chan<sup>3</sup>, Pei-Fang Lai<sup>1</sup>, Tsuey-Fong Lee<sup>1</sup>

- 1. Third Branch, Centers for Disease Control, Taiwan
- 2. Seventh Branch, Centers for Disease Control, Taiwan

3. Research and Diagnostic Center, Centers for Disease Control, Taiwan

# Abstract

On May 29, 2008, a hospital in Taichung reported a suspected case of *Entamoeba histolytica* who was a resident in a rehabilitation institution for mental disorder patients. On June 6, this case was confirmed as amoebiasis. Fecal samples from 105 residents and workers on the same floor (4<sup>th</sup> floor) were collected. Both enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) were used to test for *E. histolytica*. The results showed that 15 of the 105 people were ELISA-positive. Eight of the 15 ELISA-positives were also PCR-positive. All positive cases were residents. Workers were not infected. 9.8% (9 /92) of the residents on the 4<sup>th</sup> floor were infected. This investigation used ELISA to screen possible cases, which was at least 48 hours faster than the method of microscopy and without the bias related to a person's skill of microscopy. The results

- Received : December 12, 2008. Accepted : February 23, 2009.
- Correspondence : Min-Tsung Lin
- Address : No.20, Wunsin S. 3rd Rd., Nantun District, Taichung City, Taiwan, R.O.C.
- e-mail : mzllin@cdc.gov.tw



proved that ELISA is the best tool to screen mass cases in the initial stage of investigation. Moreover, *E. histolytica* testing must be executed before a resident admitted to a rehabilitation institution and included in residents' annual health examination in order to find asymptomatic cases early and prevent outbreak of amoebiasis in a crowded organization.

Keywords: Amoebiasis, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), stool microscopy

# Introduction

Amoebiasis is a communicable disease caused by *Entamoeba histolytica* [1]. It is an epidemic disease in most tropical and subtropical countries. It infected approximately 50 million people every year and caused about 100,000 deaths. The number of death caused by amoebiasis is only less than malaria and schistosomiasis in human's parasite infections [1,2]. However, only about 10% to 20% of infected people with clinical symptoms [3]. Currently in Taiwan, amoebiasis confirmed cases are mainly residents in home for the disabled or mental rehabilitation centers, foreign laborers, nationals returning from affected overseas areas, foreign spouses from affected countries, and gays. Most of them are asymptomatic [4-11].

On May 29, 2008, a hospital in Taichung reported a suspected case of amoebiasis, identified by microscopy. The hospital noted in the report that the case was a mental disorder patient. Third Branch of the Centers for Disease Control (CDC) notified the Public Health Bureau of the Taichung City to investigate the case and take actions to prevent and control the possible outbreak. On June 6, the Center for Research and Diagnostics confirmed the case was amoebiasis-positive. Because the case was a resident of a mental disorder rehabilitation center and this type of facilities have a high risk of amoebiasis infection, the health bureau must investigate the source and the scale of the infection. The health bureau screened the residents and workers to find out if they have been infected with *E. histolytica*. This report presents the results of this investigation and provides it as a reference for prevention and control of similar cases in the future.

#### Profile of the mental disorder rehabilitation center

The rehabilitation center mainly accommodated patients with chronic mental disorder. It was a six-story building. At the time of the investigation, there were 292 residents and 61 workers. The first floor was a multi-purpose classroom. The second floor contained a restaurant and a common room for female residents. The 3<sup>rd</sup> floor housed female residents and the 4<sup>th</sup> and 5<sup>th</sup> floors were for male residents. The 6th floor was the common room for male residents. Each ward is shared by five to 11 people with shared toilets and showers. The doors between floors were locked so residents on different floors would not be in contact with residents on other floors. Residents' daily routines started with a morning exercise. Some residents then went down to the classroom on the first floor. accompanied by staff, to participate in educational activities such as calligraphy and craft. All of the staff are Taiwanese and worked only on designated floor and do not move around between floors. This center did not employ foreign caregivers. Meals were supplied as lunch boxes by catering companies. Raw food was not supplied. Residents from each floor ate in separated areas at the restaurant. The center used tap water.



# Investigation of the outbreak

The respondents of this investigation were mental disorder patients and most of them were not able to express themselves properly, therefore most of the information was based on the description of medical staff, caregivers and residents' family members.

The index case was a 61-year-old male patient. He moved into the rehabilitation center on August 15, 2007. When he admitted, health examination was done and stool microscopic examination was negative for amoebiasis. The patient's mental condition was stable in the beginning and then developed abnormal behaviors such as eating his feces and drank his urine in April 2008. On May 1 the same year he went to hospital for teeth endodontic treatment, accompanied by his family members. Because he became mentally unstable during the treatment, he was taken into the psychiatric ward on the same day. A stool sample of the patient was tested then recorded a negative result of amoebiasis. Later, when his family tried to transfer him to another rehabilitation center, health check was done as required. A fecal sample was collected on May 28. On May 29, the result of microscopy revealed suspected of amoebiasis infection so the hospital reported this case to the CDC, as required by law. On June 6, the Center for Research and Diagnostics, CDC confirmed the test. The investigation found that all residents, including the index case, and all of the staff did not show any clinical symptoms of amoebiasis.

#### The subjects of sampling

Because the index case interacted more frequently with residents on the same floor, screening was done on residents and workers on the 4<sup>th</sup> floor to find out how many people were infected. On the 4<sup>th</sup> floor where the index case lived, there were 92 residents and 13 workers. These 105 people were the subjects of sampling and investigation. All of them were male, aged 24-77 years, with an average age of 55.6 years, and a median age of 54 years. The length of time they had lived in this center varies from 1 to 31 years, with an average of 20 years and a median of 22 years.

#### Sampling and testing methods

The original fecal sample of the index case was fixed and examined by microscopy in reporting hospital and in the next seven days, the hospital also collected fresh fecal samples three times with at least 24 hours interval between each collection. Each sample was at least the size of an adult's thumb, kept in 4°C without any fixed solution, and arrived at the Center for Research and Diagnostics, CDC within 24 hours for PCR testing. DNA of the samples was extracted. The primers were designed according to the sequences of *E. histolytica* and *E. dispar* stored in the database of GenBank. The species of *Entamoeba* was identified by PCR [9].

Fresh fecal samples collected from other residents and staffs were delivered to the laboratory, kept in 4°C.The samples were screened by ELISA kits (ProSpecT <sup>®</sup> *Entamoeba histolytica* Microplate Assay (Remel, USA)). If it was an ELISA-positive case, samples of fresh feces were collected again to perform PCR. When it was PCR-positive, the case was defined as an amoebiasis confirmed case.

# Results

The Center for Research and Diagnostics, CDC tested the first sample and suspected it as a combined infection caused by *E. histolytica* and *E* 



*hartmanni*. However, after reviewing the test result by Third Branch of CDC, and the PCR positive results of the following three fresh samples, the case was concluded as an *E. histolytica* confirmed case.

From June 10 to June 12, 105 fresh feces were collected from the residents and workers on the same floor. These samples were then sent to the Center for Research and Diagnostics, CDC on June 12 to be screened by ELISA. On June 13, 15 out of 92 residents (16%) on the 4th floor were tested positive but none of the 13 workers was tested positive. Those 15 ELISA-positive samples were then tested with PCR instead of microscopy. PCR results showed that 53% (eight out of 15) of the ELISA-positive patients were also PCR-positive, with one patient infected with both *E. histolytica* and *E dispar*. Thus, the attack rate on the residents of the 4th floor and the location of the bedrooms of the index case and residents who were tested positive. It shows that of the 11 wards, 5 of them were infected. Because residents interacted frequently in their daily life, this event could be considered as a cluster of amoebiasis.



Figure 1. The distribution of amoebiasis positive cases on the 4<sup>th</sup> floor of rehabilitation center for chronic mental patients.

# **Prevention and medical treatments**

The health bureau ordered the rehabilitation center to follow the procedures listed in the "Guidelines of infection control for psychiatric hospital (psychiatric center)" [12] to prevent and control the infection. Other than quarantining and managing infected residents and implementing disinfection, the rehabilitation center also required and supervised residents to wash hands before and after meals, and after using toilets. The health bureau also asked the center to continue monitoring residents' health, and to report to the health bureau as soon as any amoebiasis suspected cases was discovered. Treatment of the index case and other infected residents were executed by the reporting hospital and the center's contracted hospital. Paromomycin (500mg po tid for 7 days) or Iodoquinol (650mg po tid for 20 days) was used for treatments. One month after their treatment completed, all positive cases were re-tested, with three samples of fresh feces collected and tested by PCR in Center for Research and Diagnostics, CDC. When all of the test results were negative and no new cases were reported by the rehabilitation center, the investigation was concluded on September 5, 2008.

#### Discussion

Because the long latent period of amoebiasis and only microscopic examination was used before a patient admitted, the true source of this outbreak wasn't identified. Of the nine positive cases, the index case was the last one admitted to the center and his fecal sample for microscopy examination was negative for amoebiasis at admission. The other eight positive cases had lived in the center from two to more than 30 years. We



had no way to find out if all of them had been tested for amoebiasis when they admitted to the rehabilitation center. Although the center offered its residents yearly health checkup, but *E.histolytica* was not in the routine tests; it was not possible to know the sequence of infection of these nine cases. Amoebiasis can be transmitted by fecal-oral route [2,3]. Residents, including the index case, were chronic mental disorder patients. They may not have good hygienic habits because of their limited mental abilities. So, when infectious source exists, it's easy to spread and cause outbreaks.

When the index case admitted to the center, the test result for amoebiasis was negative. If this result was credible, then the index case could be infected by other residents who had moved in earlier. However, this negative result could also due to the low sensitivity of microscopy [2, 4, 13]: E. histolytica sheds intermittently in feces (it is necessary to test three consecutive sets of samples to achieve a 75% of sensitivity), or the samples tested were not enough. This explains why the hospital had only confirmed a positive result of E. histolytica after numerous samples examined under microscopy. Moreover, many asymptomatic patients will clear the infecting protozoa within 12 months [2]. Thus, the index case might have suffered from E. histolytica but admitted to the center because of his negative test result and became the source of the outbreak. However, if it's the case, then his eight roommates should be at a higher risk of being infected but only one of them was confirmed as infected case. Considering the distribution of positive cases on the same floor, it was unlikely that the index case was the source of the infection. At the beginning of the investigation, it was decided to screen only the residents and workers on the same floor, because cross-floor interactions were less frequent than same-floor. As the data of infections occurred on other floors were not available, it became another limitation of tracing the source of infection. As to the possibility of one of the workers being the source, it was eliminated because none of the workers tested positive with ELISA or came from infected areas.

Other than the fecal-oral route, drinking water or eating food, contaminated by cysts of *E. histolytica* could also contract the disease [2,3]. Gays could also become infected through anal-oral sex [7,8]. The rehabilitation center in this outbreak used tap water and consumed only boxed meals supplied by contractors. As none of the workers tested positive in ELISA, food or water contamination could be ruled out. Although this floor was occupied by only male residents, no homosexual behavior was observed. So the possibility of sexual transmission could also be ruled out. Therefore, it is still most likely that this outbreak was caused by fecal-oral transmission. When residents did not have good hygienic habit, and could not control themselves, communicable diseases spread easily.

Cases identify is one of the keys to prevent and control communicable diseases. Sensitivity, specificity, and timing of testing methods are all very important in controlling outbreaks, especially when a large number of suspected cases are pending for confirmation. Traditionally, intestinal amoeba infection is diagnosed by direct microscopic examination for trophozoites or cysts based on morphology. Haematophagous trophozoites is the diagnostic criteria for invasive amoebiasis. *E. histolytica* sheds into feces intermittently. 75% of sensitivity can only be obtained if three fresh feces are collected in 7 days with intervals of at least 24 hours. More

samples may be required and the tests must be done by experienced technicians with a lot of time. Unstable sensitivity and time consuming are the major disadvantages [2,4,13]. Using ELISA to screen samples avoid the above limitations. ELISA method, only one fresh sample is required, is 48 hours quicker than traditional microscopy method and does not require a high level of equipment or experience. It does not need specially trained technicians so more samples can be processed and can minimize the problem of bias caused by technicians. Although ELISA can detect the common antigen of *E. histolytica* or *E. dispar* in fresh feces, like microscopic method, it can not distinguish them and still requires PCR to confirm if the case is infected by *E. histolytica*. PCR has a higher sensitivity and specificity than ELISA. But because PCR requires more complicated skills and equipments, higher cost, and takes longer time [14], ELISA is still a better option for large-scale screening.

This investigation did not re-test those ELISA-negative cases with PCR so the sensitivity and specificity of this screening is not known, which is another limitation of this study. However, Ong et al. used both ELISA and microscopic methods to screen *E. histolytica* in a psychiatric hospital in Eastern Taiwan in 1996 [4]. Their results showed that the sensitivity and specificity of ELISA was 78% and 99%, respectively, and the detection rate was 1.5 times higher than microscopy (16.9% vs. 10.9%). In 2001, Deng et al. used the ELISA method to screen amoebiasis in an institution for the disabled located on Southern Taiwan; 38 of 442 people were tested positive. Using PCR to check again, fifteen people were tested positive. The positive predictive value was 39% (15/38). All tested positives were asymptomatic [9]. In our investigation, the positive predictive

ncon

value was 53% (8/15).

For the samples collecting from the contacts of confirmed amoebiasis cases, fresh feces from those contacts with symptoms were collected three times in seven days, (with a minimum interval of 24 hours), and processed for PCR testing in research and diagnostic center, CDC. Samples collected from those without symptoms were collected in the same way, but mixed and stirred with merthiolate-iodine-formaldehyde (MIF) solution before being tested by microscopy in local health bureau. If any trophozoites or cysts were found in any of the three fecal samples, then three more fresh feces would be collected. These three new samples, along with the three original fixed samples, would be tested by PCR for confirmation in research and diagnostic center, CDC. If the confirmed case is a resident of an institution for mental disorder or handicapped persons, and there was a concern of further spread of the disease, local health bureau could ask CDC to help screen everyone in the institution, using ELISA. In this event, the index case was a mental patient. So Third Branch of CDC asked the Public Health Bureau of Taichung City to execute the investigation and implement relevant control and prevention procedures immediately. Three issues emerged when the index case was confirmed positive. First, the index case had some abnormal behavior such as eating his feces. Second, psychiatric hospitals and rehabilitation centers are high risk environments of amoebiasis outbreaks. Third, there were at least 105 people needed to be screened immediately. Using the traditional microscopic method would be very inefficient and would compromise the timeliness of the prevention and control of this outbreak. Third Branch of CDC contacted the Center for Research and Diagnostics and asked using ELISA method to shorten



the time needed and reduce errors by microscopy examination. It was not unusual to miss detecting contacts infected *E. histolytica* in previous investigations because of the low sensitivity of the microscopic method and inexperienced examiners. Thus, in May 2008, CDC called a conference to evaluate the procedure of amoebiasis prevention and control. Sampling procedures for contacts of confirmed cases were modified. Regardless the existence of clinical symptoms, all samples must be tested by ELISA first. If a case tested positive, three fresh fecal samples will be collected and send for PCR confirmation [11]. However, when this event occurred, the health bureau in charge had not begun the purchase process of ELISA test kits so it asked for the help from CDC. It shows how important ELISA is in screening *E. histolytica*.

In the past, remote districts, as well as Changhua, Nantou, Yunlin counties in Middle Taiwan had been high risk areas of amoebiasis outbreaks. Nowadays, only remote districts still have higher positive rate because of significant improvement of environmental hygiene [6]. Confirmed cases are mainly foreign laborers and spouses from infected areas, Taiwanese citizens returning home from infected areas, gays, and residents in mental hospitals or handicapped centers. Some reports have pointed out that clusters of amoebiasis in Taiwan mostly happened in psychiatric facilities [4-6,9,10] and their residents are at a high risk of contracting amoebiasis. In order to avoid communicable disease outbreaks happening in rehabilitation centers and other similarly organizations, Taiwan's CDC has published the *Guidelines of infection control for psychiatric hospital (psychiatric center)* and the *Guidelines of infection control for institution* since Nov 2001 [15]. The guidelines suggested that organizations should

# Taiwan Epidemiology Bulletin

take the following actions to avoid outbreaks of infectious diseases: Health evaluation must be done at the time when a resident admitted. If the resident has any communicable disease and needs isolation, an applicable room must be provided and the patient should be transferred to a hospital if further treatment is required. In the week before admitted, the new resident must submit negative test results of shigellosis, amoebiasis, and parasite infections or to be isolated for a week to make sure there were no symptoms related to gastrointestinal infectious diseases before the patient is allowed to move into general wards. In addition, to prevent outbreaks, institutions should also routinely screen all residents for amoebiasis in order to understand residents' health condition and to find and treat asymptomatic cases.

## Acknowledgements

Sincere thanks to Dr. Dar Der Ji at the Research and Diagnostic Center for his assistance in ELISA; to Miss Hung Chung Chen in the Public Health Bureau of the Taichung City and to Miss Mei Hui Tsai, nurse supervisor for supplying the information of the rehabilitation center. Thanks for their most invaluable help to this investigation.

#### References

- WHO/PAHO/UNESCO report. A consultation with experts on amoebiasis. Mexico City, Mexico 28-29 January, 1997. Epidemiol Bull 1997; 18: 13-4.
- 2. Van SJH. Amoebiasis: current status in Australia. Med J Aust 2007; 186: 412-6.
- 3. Stanley SL. Amoebiasis. Lancet 2003; 361: 1025-34.
- 4. Ong SJ. Use of the 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: 248-9.

- Chao DY, Wu PF, Chen KT. Investigation of an entamoeba histolytica infection in a provincial institution. Taiwan Epidemiol Bull 1997; 13; 135-144.
- Jiang DS, Chang KH. An investigation of amebiasis outbreak in one rehabilitation center for mentally retarded children. Public Health Quarterly 2000; 26: 261-270.
- Hung CC. Invasive amebiasis as an emerging parasitic disease in patients with human immunodeficiency virus type 1 infection in Taiwan. Arch Intern Med 2005; 165: 409-15.
- Hung CC. Increased risk for entamoeba histolytica infection and invasive amebiasis in HIV seropositive men who have sex with men in Taiwan. PLoS Negl Trop Dis 2008; 2: 175.
- Deng HY, Hsiao WH. Epidemiological study of amebiasis and strain analysis of pathogenic amoeba in an education and nursing institute for the mentally- handicapped in Taiwan. Taiwan Epidemiol Bull 2005; 21: 1-14.
- Chen YH. Outbreak of amoebiasis in a psychiatric hospital Kaohsiung County. Taiwan Epidemiol Bull 2008; 24: 780-9.
- Taiwan CDC. Communicable disease control manual. Amebiasis. Available at: http://www.cdc.gov.tw/public/Attachment/87181764271.pdf.
- Taiwan CDC. Guidelines of infection control for health care organization. Guidelines of infection control for psychiatric hospital (psychiatric center). Available at: http://www. cdc.gov.tw/public/Attachment/99710412771.pdf.
- Fotedar R. Laboratory diagnostic techniques for *Entamoeba* species. Clin Microbiol Rev 2007; 20: 511-32.
- Tanyuksel M, Petri WA. Laboratory diagnosis of amebiasis. Clin Microbiol Rev 2003; 16: 713-29.
- Taiwan CDC. Guidelines of infection control for health care organization. Guidelines of infection control for institution. Available at: http://www.cdc.gov.tw/public/Attachment/ 9989294171.pdf.