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Investigation of an *Entamoeba histolytica* Infection in a Provincial Institution

1. Introduction

Amebiasis is a disease of the large intestine due to the invasion of *Entamoeba histolytica*. Infection is mainly by soiled hands, contaminated water or food, or direct contact with carrier containing cysts of the protozoa. Worldwide, the infection rate is around 12% [1-5].About 90% of the infected are asymptomatic and become carriers. Clinical symptoms include: general gastro-intestinal symptoms, diarrhea. enteritis and amebomas. Around 2-20% will develop direct extension to produce liver abscess [1-5].

On 20 February 1995, upon report of the Tainan County Health Bureau that a student of a care institution was diagnosed as dysentery by the National Cheng-kung University Hospital, the Taiwan Provincial Health Department instructed the Bureau for investigation and disease control. Preliminary investigation of the Bureau showed that the care institution had a total of 447 retarded children and 186 staff members: and that with the exception of the patient, no other members of the institution showed any symptoms. On 23 February, laboratory technicians of the Taiwan Provincial Health Department, and staff of the Bureau of Communicable Disease Control and Division of Malariology and Parasitology of the National Institute of Preventive Medicine, both of the Department of Health, visited the institution for stool collection. To understand the epidemiology of the outbreak. FETP members also visited the institution on 28 February.

Upon investigation, it was noted that a student of the institution died of liver abscess in the National Cheng-kung University Hospital in January. The student was admitted to the Taiwan Provincial Hsinying Hospital in December 1994 for high fever. He was later transferred to the National Cheng-kung University Hospital for treatment of liver abscess. The tissue culture was confirmed liver abscess. Later, the condition worsened, and the patient developed complications of epilepsy and dyspnea and eventually died. In February 1995, another student developed similar symptoms and was admitted to the hospital. On 22 February, the institution asked the local Chiu Hospital to examine by IHA method students who either shared the same dormitory or went on an excursion the previous month with the two students. Of the 157 students examined, 49 were found

positive, giving a positive rate of 35%.

Water supply of the institution comes in two systems: the tap water for drinking and cooking, and the underground water for washing and other cleaning purposes. The underground water is treated in a way as a simple water supply system through filtration, sedimentation, air-exposure and chlorination before use. Waste water is disposed untreated through covered drainage. Food is prepared by cooks of the institution. Food is brought to the dormitory and shared in the dormitory dinning room.

2. Method

1. Questionnaire Survey:

A structured questionnaire was used. The questionnaire contained items such as: the background information, symptoms, and any infection of amebic dysentery in the past. It was administered to 633 staff members and students. As the students were either seriously retarded or disabled, their questionnaires were filled out by caretakers.

A suspected case was defined as: any person of the institution (student or staff) who had diarrhea between December 1994 and February 1995 and also one of the following symptoms:

(1) abdominal discomfort
(2) nausea
(3) diarrhea alternating with constipation
(4) stool with pus
(5) stool with mucus

The questionnaires were treated with D-Base Ill Plus and analyzed with Epi-info V6.O.

2. Laboratory Testings:

Stool specimens were collected from all staff and students and tested by MIF (methiolate-iodine-formalydehyde) method.

On 1 March, blood specimens of all staff and students were collected for testings by IHA (indirect hemagglutination test) method.

3. Environmental Specimens:

Samples of filtered underground water, underground water, tap water in the kitchen, waste water in the drainage, water used in the patient's dormitory and waste water in

toilets were collected for testings by the Tainan County Health Bureau.

4. Medical Records:

A physician visits the institution once every week. Records of the visits were studied to see if there was any indication of gastro-intestinal diseases.

3. Results

1. Questionnaire Survey:

All of the 447 copies of the questionnaire distributed to students were returned, giving a return rate of 100%; of the 186 copies distributed to staff members, 172 were returned, giving a return rate of 92.5%. 20 of the students, 15 males and 5 females, met the criteria for a case, giving an attack rate of 4.5%; none of the staff met the criteria, giving an attack rate of 0%. Table 1 shows the distribution of cases and attack rates by dormitory: one case each in dormitories 1, 2, 3, 8 and 9; two cases each in dormitories 5 and 7; four cases in dormitory 4; and seven cases in dormitory 6. Symptoms included: diarrhea (20 cases, 100%), abdominal pain (7 cases, 35%), vomiting (2 cases, 10%), and diarrhea alternating constipation (4 cases, 20%).

Table 1. Distribution of Cases by Dormitory

Dormitory	Total No.	No. of Cases	Attack Rate (%)
1	50	1	2.0
2	35	1	2.9
3	50	1	2.0
4	50	4	8.0
5	50	2	4.0
6	50	7	14.0
7	50	2	4.0
8	48	1	2.1
9	64	1	1.6

2. Laboratory Testings:

1) Fecal Examinations:

68 were found positive: 15 in dormitory 1, 2 in dormitory 2, 5 in dormitory 3, 16 in dormitory 4, 8 in dormitory 5, 6 in dormitory 6, 8 in dormitory 7, 6 in dormitory 8, and 2 in dormitory 9, giving an attack rate of 15.2% (Table 2). By type, 3 (4.4%) of the 68 were of the active type, 6 (8.8%) of the cyst type, and 59 (86.8%), the combined type.

Table 2. Findings of Fecal Examinations

Subjects examined	Total No.	No. Positive	%
Students in Dorm 1	50	15	30.0
2	35	2	5.7
3	50	5	10.0
4	50	16	32.0
5	50	8	16.0
6	50	6	12.0
7	50	8	16.0
8	48	6	12.5
9	64	2	3.1
Total	447	68	15.2
Staff	186	0	0.0

2) Testings for Sera-Antibodies:

At an antibody potency value of 1:256, 130 students were found positive in the IHA sera-antibody tests, giving a positive rate of 29%. None of the staff was positive. Table 3 gives the distribution of positive rates by dormitory.

Table 3. Distribution of IHA Positive Case by Dormitory

Dormitory	Total No.	No. Positive	Positive Rate (%)
1	50	26	52.0
2	35	6	17.1
3	50	11	22.0
4	50	16	32.0
5	50	17	34.0
6	50	16	32.0
7	50	18	36.0
8	48	17	35.4
9	64	3	4.7
Total	447	130	29.1

By using χ^2-test corrected with Fisher's Exact 2-tailed Test to test the difference in positive rates between cases and non-cases, no difference was found ($p > 0.05$) (Table 4). This could mean that the diarrhea of cases was probably not associated with amebic dysentery. Testing by x2-test of infection and IHA positive rates did not show significant difference either ($p > 0.05$) (Table 5).

Table 4. Relation Between Suspected Cases and Fecal Examination

	Fecal Exam Positive	Fecal Exam Negative	p-Value
Cases	4	64	0.52*
Non-cases	16	363	

* Fisher's exact test

3) Environmental Specimens:

Testings of environmental specimens by the Environmental Protection Bureau for E. coli and coliform all showed negative.

Table 5. Suspected Cases and Sera-Antibody Tests

	Sera-antibody (+)	Sera-antibody (−)	p-Value
Cases	8	12	0.27*
Non-cases	122	305	

4) Medical Records:

Monthly records prepared by the health room of the institution did not show any significant outbreak or clustering of gastro-intestinal diseases in 1994 (Table 6).

Table 6. Cases of Gastro-Intestinal Diseases in 1994

Month	Dormitory									Total
	1	2	3	4	5	6	7	8	9	
Jan	5	6	1	7	4	1	3	1	5	33
Feb	4	1	2	14	5	2	1	2	5	36
Mar	3	1	4	4	2	5	4	2	4	29
Apr	2	0	5	2	7	4	3	1	13	37
May	1	1	2	5	9	4	7	3	12	44
Jun	2	4	1	2	1	2	0	0	7	19
Jul	0	1	0	4	5	3	3	0	8	24
Aug	3	1	6	10	0	1	2	1	4	28
Sep	1	3	1	5	1	2	8	0	4	25
Oct	6	3	0	4	2	5	2	3	7	32
Nov	1	0	1	0	3	3	1	2	3	14
Dec	2	0	5	3	3	4	2	7	4	30

4. Discussion

Amebiasis is the third cause of death in all parasitic infectious diseases. Each year in the US, around 3,500 cases of amebiasis are reported to the Centers for Disease Control and Prevention [(2)]. Reports show that tourists, immigrants, migrant workers,

persons with inhibited immune system, psychiatric patients in institutions and male homosexuals are more vulnerable. Asymptomatic carriers are often major hosts of *Entamoeba histolytica* protozoa [1].

Diagnosis of amebiasis is traditionally by identifying through microscopes trophozoites or cysts of *Entamoeba histolytica* in feces or sections from lesions. Reports, however, show that even after six to nine examinations, the identification rate is only 72-74% [4], and that there often are either false negative or false positive identifications. False negative identification is the result of poor specimens collection or poor skills; and false positive is due to the interference with white blood cells, blood platelets or other protozoa [2, 3]. In the case of *Entamoeba histolytica* invading outer intestinal tissues, microscopic examination of feces is unlikely to detect the protozoa, serological methods such as IHA (indirect hemagglutination test) are more useful. However, IHA test could give false negative reading when antibody potency is low and cases may thus be lost; or, in endemic areas, sera-antibody potency of the population is generally higher due to high prevalence, and as antibody potency can last for some time, it makes the differentiation between new and old cases difficult [1].

The sensitivity of detecting cases as defined by the present study through fecal examination was 5.9% (4/68); and the specificity being 95.8% (363/379) (Table 4). The reason is that almost 90% of the amebiasis infections are asymptomatic. Findings of laboratory testings and questionnaire survey of the 68 cases showed a relatively high positive rate of IHA antibody. This could mean that the infection was caused by chronic carrier. While the environmental specimens were all negative, and only students were infected, the infection was not likely to be associated with either the food or drinking water of the institution, as the monthly bill for public water supply was as high as NT$5-60,000. The monthly medical records of the institution showed that in 1994, on average 29 students were treated for gastro-intestinal diseases. This figure corresponded to the 20 cases identified by the questionnaire survey. Thus, the present suspected amebiasis outbreak of the institution was most likely an incident of amebic liver abscess. The prevalence of positive rate by microscopic examination of feces in the present incident was 15.2%. Surveys of the US CDC showed that, worldwide, the prevalence rate of amebiasis was around 10% [6]; the prevalence rate found in some Japanese institutions for retarded children was 12.6% [7]. In serological testings, the prevalence rate of amebiasis in Mexico as a whole was 8.41 % [8]; that in the Orchid Island found in a study by the National Institute of Preventive Medicine was 42.58% [9]; and that in the present study was 29%. In other words, in the high prevalence rate of amebiasis throughout the world, the institution was no exception. The actual number of amoebic dysentery cases in the present incident should be four (Table 4).

The two test methods, the fecal examination and the IHA test, were found highly related ($p<0.0l$) (Table 7). That is, a case identified positive in the fecal examination would also be positive in his/her sero-antibody to amoebic dysentery. There could be two reasons: one is, a person who was at one time infected with invasive amebiasis is presently only a carrier of cysts; the other is, a person who was recently infected with invasive amebiasis is presently a carrier of cysts. Though there is no further evidence to

support this deduction, it is noted from literature that in a follow-up epidemiological survey in Mexico of chances of asymptomatic carriers of amebiasis cysts and their family members developing invasive amebiasis for instance, it was found, after eight months of follow-up, that 40% of the family members were infected; whereas the carriers remained asymptomatic, though 27% of them were found by Zymodeme method to have developed invasive amebiasis [10]. Carriers of amebiasis cysts though often remain asymptomatic, they could develop invasive amebiasis and show positive in their sero-antibody to amebiasis.

Table 7. Relation Between Fecal Examination and IHA Test

	Sera-antibody (+)		Sero-antibody (−)		
	Positive	Negative	Positive	Negative	p-Value
Fecal examination	53	77	15	302	0.001

It is noted from literature that from persons who have had amebic liver abscess or symptomatic amebic protozoa of the intestinal tracts, IHA test is capable of detecting IgG and IgA in their sera [11]. However, in an endemic area of amebiasis, for the high sensitivity and low specificity of IHA, the positive cases detected by IHA can only mean that they have been infected at one time. As there is also cross reaction with other protozoa infections, there is no way to prove that the cases have been infected either recently or some time before. Many diagnostic methods have been suggested. In the US the western block method has been used to detect serine-rich *Entamoeba histolytica* protein in antibodies to differentiate acute invasive amebiasis and old infections [12]. Zymodeme method has been used to differentiate invasive and non- invasive amebic protozoa [13]. These methods, however, are more expensive than the IHA method. As microscopic examination of feces may lose 30-40% of clinically suspected cases, it has been suggested in literature that ELISA method be used in the detection of amebic antibody in feces. When the two fecal examination methods are compared with the capture-recapture model, the sensitivity of the centrifugalization method is 100%; and that of the MW direct microscopic method is only 44%. Though the often used MIF direct microscopic method can miss many positive cases, for its convenience and prompt results, it is still an effective tool in the investigation of outbreaks.

5. Recommendations

1. Institutions like this one are likely to transmit infectious diseases [1]. Feces of students or patients, if not treated, can be easily spread through drainage to the neighboring communities. The institution is recommended to treat waste water before disposal.

2. Treatment including isolation and medication with anti-protozoa medicines such as 5-Metronidazozle, lodoquinol or Furanmide should be given to the 68 amebiasis positive students.
3. Specimens should be collected from families of positive cases and health education given to them to avoid any further infections.
4. Ii was noted in the present investigation that IHA method in outbreaks such as this was not able to differentiate either current or previous infections. Fecal examination was therefore still considered an effective method.
5. In handling similar outbreaks like this, the media should be warned not to make premature reportings to avoid any panic and harms to the students and the institution.

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