Overview of *Entamoeba histolytica* and Amebiasis in Korea with Special Reference to Research Notes

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**Abstract:** Amebiasis caused by *Entamoeba histolytica* infection has been a prevalent protozoan disease in Korea since old times. For this reason, a number of scientific publications in relation to this protozoa has totaled 186 so far in the literature, as of 1989. The subjects ranged from biology to its treatment and prevention.

The overall achievement by scientists and clinicians in Korea has been meritorious in elucidating some of the pending problems in the areas of pathogenesis, diagnosis, and treatment. Among them, development of a culture method applying diphasic medium, studies on virulence of the strains collected in Korea, and a combination of aspiration method and chemotherapy to treat amebic liver abscess are seen to have been contributing to the eventual goal of reducing this protozoa infection from the land.

**Key words:** *Entamoeba histolytica*, overview, hepatic amebiasis, epidemiology, pathogenicity, diagnosis, treatment

**AMEBIASIS IN KOREA**

**Historical outline:**

Historical information on amebiasis in Korea is rather limited in spite of its high prevalence. It is said that the term “E-Jil” or “Kojuri”, which denotes dysentery, was introduced from China, probably during the Ki-Ja dynasty around 1,000 B.C. The first mention of “E-Jil” appeared during the Koryo dynasty (918～1392AD), namely that King Shin Jong (1197～1204) suffered from “E-Jil” in 1202A.D. (Chun, 1975). Je-Ma Lee, born in 1836 A.D., made several recommendations on the treatment of dysentery in his book titled, “Dong E Soo-Se Bo-Won”. However, no scientific criterion to define the cause of dysentery was employed until the microscope was introduced into Korea. That was probably the late part of the 19th century. Dr. H.N. Allen, a missionary of the Presbyterian Board of Missions, saved the life of a nobility. In gratitude, King Ko-Jong permitted Dr. Allen to open a hospital, Kwang Hei Won, in April 1885. This became the first hospital for western medicine in Korea. The microscope was supposedly utilized in this hospital in order to examine pathogenic agents. In medical literature, Shiga (1991) was the first to describe dysentery with scientific knowledge, and several extensive reports followed.

According to literatures two kinds of dysentery have been known in Korea, namely, bacillary and amebic. Hitherto, amebic dysentery was believed to be more prevalent in Korea than dysentery of bacillary origin, although scientific data suggest that prevalence of dysentery of amebic origin is much lower than that of bacillary origin. Yamaguchi (1913) classified the dysentery cases in Government Hospital in Seoul and found that amebic dysentery cases were far less frequent than those of bacillary origin. In 1912, he studied 110 cases of dysentery and found that 93 (81.5%) were of bacillary, and only 17 (15.5%) of amebic etiology. Analysing 251 dysentery cases
Table 1. Incidence of bacillary and amebic dysentery (Chun, 1975)

<table>
<thead>
<tr>
<th>Reporter</th>
<th>Place</th>
<th>Year</th>
<th>No. of dysentery cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takida</td>
<td>Countrywide</td>
<td>1926~1930</td>
<td>5,188 20</td>
</tr>
<tr>
<td>Sato</td>
<td>Countrywide</td>
<td>1911~1912</td>
<td>198  45</td>
</tr>
<tr>
<td>Matsumoto</td>
<td>Daegu</td>
<td>1911</td>
<td>14  3</td>
</tr>
<tr>
<td>Ishihara &amp; Sano</td>
<td>Seoul</td>
<td>1927~1931</td>
<td>246 14</td>
</tr>
<tr>
<td>Subia &amp; Chiba</td>
<td>Seoul</td>
<td>1934~1938</td>
<td>434  6</td>
</tr>
<tr>
<td>Kim &amp; Seuk</td>
<td>Seoul</td>
<td>1939~1946</td>
<td>1,131  25</td>
</tr>
<tr>
<td>Moon et al.</td>
<td>Wonju</td>
<td>1964</td>
<td>5/253* 38/253*</td>
</tr>
<tr>
<td>Chang et al.</td>
<td>Busan &amp; Namsan</td>
<td>1964</td>
<td>0/711* 37/711*</td>
</tr>
</tbody>
</table>

* diarrhea cases

In 1913 (January~October), he found 231 (91.6\%) were bacillary and 20 (7.8\%) of amebic. However, it should be kept in mind that *Entamoeba histolytica* causes not only dysentery, but also various types of gastrointestinal troubles and extra-intestinal diseases.

Regarding extra-intestinal amebiasis, Moriyasu (1913) first reported liver abscess formation as a complication of amebic dysentery. Subsequently, Ludlow (1926) diagnosed a greater number of liver abscess cases for a decade and elucidated more clearly the pathogenicity of the protozoa in liver abscess in Korea. During the years of 1927~1934, a number of studies concerning culture method, pathogenicity, epidemiology, and treatment were performed by Chiba (1931), Kuwabara (1930), Nagahama (1935), and their coworkers. Regarding prevalence, it is presumed that the number of actual amebiasis cases may be greater than the reported.

In general, amebiasis shows rather mild but insidious symptoms as compared with bacillary dysentery and is chronic rather than acute. For this reason the patient usually finds medicine from a herbist or drugstore instead of visiting a modern clinic. Such factors may bring about the statistically lower incidence of amebic dysentery than of bacillary.

**Intestinal amebiasis:**

In spite of its high prevalence there are little formal statistical data on intestinal amebiasis. The symptoms may vary from asymptomatic to fulminant dysentery characterized by diarrhea with blood and mucus in the stool. In this chapter, clinical cases only will be reviewed. Yoon and Choi (1966) analyzed 85 cases of amebic dysentery in children. They found that among 70 cases the incidence was higher during the summer months (January: 5, February and March: 1 each, April: 2, May: 3, July: 4, August: 8, September: 16, October, November and December: 7 each). The initial manifestation was diarrhea in all cases associated with fever 70%, vomiting 36.5%, and dehydration 35.4%. Stool examination disclosed blood and mucus from the onset in 38.8% and mucus only in 27%. The stool frequency ranged from 6 to 10 times per day in 76% of all the patients. Among physical findings, leucocytosis (51.8%), generalized edema (25.9%), and hepatomegaly without abscess (3.5%) were observed in that order. Intestinal amebiasis occasionally develops to the point where surgical intervention is indicated. Yun (1968) reported one case of amebic granuloma. The case was a 60-year old female who had previous complaints of tenesmus or colitis. On examination, a tumor of child fist size was palpated at 7 cm above the anus. The surface was eroded, and *E. histolytica* trophozoites were isolated from the submucosa following biopsy. He also reported that other types of surgical amebiasis such as cecal amebiasis, amebic anal fistula, and perforation of the colon were not uncommon in clinics.

Byoun and Hong (1968) also analyzed the physical sign and clinical finding in 48 cases
of amebic colitis who were admitted to St. Mary’s Hospital in Seoul during 5 consecutive years beginning in 1963. In all cases with *E. histolytica* positive stools were included. The patients complained of clinical symptoms, but were varied. Children complained more of diarrhea, abdominal pain with distention and dehydration, whereas the adult group showed nutritional deficiency, anemia, diarrhea and neurological symptoms. The more frequent symptoms were diarrhea, abdominal pain, mild fever, and nausea in that order. Tenderness in the lower abdomen was noted in about half of the cases. Sigmoidoscopy examination disclosed ulcerative changes, mostly in the descending and sigmoid colon in 50%. About one-third of the cases complained of mild fever, 37% had leukocytosis, and the erythrocyte sedimentation rate increased in 21%. Complications were liver abscess (12.5%), intestinal perforation (2.1%), empyema (2.1%), and arthritis (2.1%). Antiamebic treatment resulted in satisfactory outcome in all the cases, except for one with a fatal outcome due to intestinal complications.

**Hepatic amebiasis:**

Hepatic involvement in *E. histolytica* infection is frequently considered to be a complication of intestinal amebiasis and is relatively common in Korea. Even though the pathological criteria have not yet been detailed, the clinical manifestations can be divided into 2 types: hepatic enlargement and hepatic abscess.

**(1) Hepatic enlargement**

Amebic hepatitis is known as a form of early regional hepatic necrosis associated with massive invasion of the affected area by *Entamoeba histolytica*. Thus, I will define hepatic enlargement as a hepatitis state, since no definite diagnosis of amebic hepatitis without abscess has been reported by any autopsy. A few reports on amebic hepatitis or hepatomegaly in Korea are available. Lah (1960) analyzed 25 clinical cases, and thought to have amebic hepatitis by symptomatic pattern and good response to antiamebic drugs. The main and most frequent subjective symptoms were dyspepsia (60%), upper abdominal pain and/or discomfort (60%), constipation (52%), low grade of fever (36%), diarrhea (32%), right upper quadrant pain (28%), vomiting (20%), nausea (12%), and headache (12%). Objective-ly, hepatomegaly with tenderness and colonic thickening, as well as swelling with tenderness, were invariably seen. Laboratory examination showed mild leucocytosis (ranging up to 19,000/mm³, average of 8,800/mm³) and almost normal liver function tests (only one case with abnormal B.S.P. retention, 3 cases with clinical jaundice). Cho et al. (1967) carried out an extensive study on Jeju Island where pigs were raised with human nightsoil and open-well was used for drinking and laundry. A total of 738 fecal specimens were examined during the years of 1965~1966 with an average percentage of 24.3% for *E. histolytica*. Under such conditions, hepatomegaly cases in 2 villages, Sinwom-Ri and Yongheung-Ri, were examined. In Sinwom-Ri, 17 (11.3%) individuals had hepatomegaly among the 150 examined, and over half of them were positive for *E. histolytica* cysts in feces. In Yongheung-Ri there were 79 (37.1%) hepatomegaly cases among the 213 examined. The hepatomegaly cases showed 59.7% *E. histolytica* cyst positives by fecal examination. In Sinwom-Ri hepatic enlargement in males was more than 3 times as frequent as in females, and yet there were no significant differences in incidence according to sex and age. Among 61 hepatomegaly cases, 54% had a history of diarrhea within the past several years, and about 10% had experienced some kind of hepatic disorder.

At the time of physical examination, the patients complained of various general symptoms such as fatigue, headache, shoulder pain, and so on. The main gastrointestinal symptoms were nausea, capricious appetite, diarrhea or soft stools, and abdominal pain in decreasing order. Eighty-three percent showed soft hepatic margin, and about 44% complained of tenderness in the liver. Urobilinogen test and cephalin-cholesterol flocculation test were 23.8% and 45.6% positive, respectively. The overall findings suggested that hepatomegaly is presumably related to infection
with *E. histolytica*. Amoebas might be introduced into the liver via portal vein from the intestinal foci. Fifty-four percent of the cases had a history of diarrhea or mucoid stool during the previous year, and 6.8% of them suffered from certain hepatic disorders. Thus, hepatic enlargement is most likely related to intestinal amebiasis.

(2) Hepatic abscess

A considerable number of amebic liver abscess cases has been reported in Korea since Ludlow (1923). Roh and Kim (1948) analysed 159 cases of amebic abscess admitted to Severance Hospital during 1921–1947. The age range was 21–50 years and 140 were male. Thus, males predominated in incidence. It is probable that the higher rate of alcohol consumption in the males provided favorable conditions for amebic invasion of the liver. They found that 83.7% of the 159 cases were alcohol consumers, and 116 cases confessed a past history of dysentery. Min (1971) also examined the charts of the 178 cases of liver abscess who were admitted to Severance Hospital during the years 1955 to 1970. According to etiological classification, 102 cases (57.3%) were of amebic origin and 76 cases (42.7%) of pyogenic origin. Amebic abscess was diagnosed by symptoms, parasitological examination, nature of the pus, and drug response. *E. histolytica* trophozoites were found in only 16 cases (15.7%) out of 102, but even in the negative cases the chocolate-brown color and anchovy-paste nature suggested amebic origin. Among the 102 cases, 85 or 83.3% were male and 17 or 16.7% were female. Peak incidence was noted in the 30–40 years age group, ranging from 30.4% to 32.4%. The main symptoms were pain in the right upper quadrant in 85.3%, then fever and chills followed. Jaundice was noted in 6 cases. Leucocytosis of 10,000–20,000 and above was found in 75.5%. Ninety-four cases (92.1%) had right lobe involvement, and only 6 cases (5.9%) left lobe involvement. Roh and Kim (1948) also reported that in 154 out of 159 amebic abscess cases the abscess was in the right lobe, and leucocytosis was 11,035 on the average. For etiological diagnosis, bacteriological and parasitological examinations should be done in order to differentiate amebic and pyogenic abscess. In such a confusing case serological tests are recommended as a supportive procedure.

**Pulmonary amebiasis:**

About 10~20% of the liver abscesses were complicated by pulmonary abscess (Kim, 1963), whereas primary pulmonary amebiasis is recognized as relatively rare in Korea. Kim (1963) reported 9 cases of pulmonary amebiasis, one primary and 8 secondary. He detected the ameba in 60~75% of patient supta.

Liver abscess ruptures and ameba in the pus may spread to the surrounding areas. Ludlow (1923) observed it in 20 out of 100 amebic liver abscess cases: in 9 cases to thoracic cavity, in one case to the thoracic wall, in 5 cases to the bones of the rib area and in 5 cases to the abdominal cavity. Due to its anatomical position the majority of the abscess is formed in the upper part of the right lobe of the liver, and the amebae reach the thoracic cavity by passing through the diaphragm. The main complications of amebic liver abscess were pericarditis (Kim, 1964; Park et al., 1966) and lung abscess (Choi, 1968).

**Other extra-intestinal amebiasis:**

It is conceivable that there might exist extra-intestinal amebiasis other than liver and lung abscess, but only a few reports are available in the literature: 3 cases of amebiasis in uterine cervix and vagina by Lee et al. (1965); 5 cases of amebic pericolicitis together with one case of coxitis and one case of amebic psoitis by Kim (1962); and one case polyarthritis due to amebic colitis by Kim et al. (1968). Metastasis to the brain, kidney, spleen, oesophagus, ovary, testis, and skin have been reported elsewhere, but no references are available from Korea.

**Epidemiological Notes**

**Prevalence:**

*Entamoeba histolytica* has been a commonly
known pathogenic species among the protozoa in the country. Kessel (1925) found a 41% prevalence by 4 successive direct smears among 208 examinees, and Choi (1926) reported 1.5% positive among 2,000 fecal samples by single direct smear, whereas 30.2% in Seoul area were positive after 6 repeated examinations. Chiba (1931) also obtained similar results. Soh et al. (1961) reported 4.3% of 10,320 fecal samples to be positive for *E. histolytica* and found no special age or seasonal differences among them, but Yoon and Choi (1966) reported that clinical cases were predominant during the summer.

Kim et al. (1971) reported 6.4% prevalence by direct smear method, zinc sulfate flotation method, or formalin-ether concentration method among 2,250 fecal specimens which were collected from 10 localities in different provinces. The overall figures showed that prevalence ranged between 3.3% and 9.9% and the national average was 6.4%. But the prevalence rates varied according to reporter, examination method, and area even within the same province. Nevertheless, the general trend in recent years has been one of declining incidence rates compared to several decades ago. Environmental factors and traditional habits of the inhabitants may cause in maintaining some constancy.

**Age and sex:**

In a nationwide survey of the cystic stage of *Entamoeba histolytica*, Kim et al. (1971) found higher prevalences of 8.9% in the 50 years and above age group. Kim (1967) reported a high prevalence in the 31-60 years of age group with rates of 31.6-34.6%. On Jeju-Do, Yoon and Choi (1966) found that infants less than 2 years old already suffered from amebic dysentery in 28 cases (33%) out of a total of 85 below 15 years of age.

Prevalence of *E. histolytica* infection seems to be higher in females than in males (Soh et al., 1961). The data by Kim et al. (1971) show the ratio as 7.5 : 5.3. Kim (1967) analyzed 211 positive cases on Jeju-Do and found 22.1% to be male and 26.9% female. Yoon and Choi (1966) examined 85 cases of amebic dysentery among children and found that females exceeded males. On one side, Kim (1971) analyzed the 102 amebic liver abscesses on Jeju-Do and found 83.3% to be male and only 16.7% was female. Although no logical explanation can be offered yet, environmental factors, habits, and differences in physical conditions of each sex may be contributing causes to such diversities.

**Occupation:**

The occupations of the Korean people differ somewhat from those found in western countries, and the conditions under which they live vary according to financial status and educational standard. No recent data according to occupation are available. Nevertheless, old literature (Sato, 1913) indicates that a specific relationship between occupation and disease occurrence can not be established, and is rather diverse. For example, laborers, artisans, carpenters, and low-level technicians usually showed a higher incidence compared to economically stable classes.

**Seasonal variation:**

Infection with *E. histolytica* is chronic and insidious in nature. For this reason, it is not logical to draw conclusions based on seasonal variations. From an ecological viewpoint, however, it is estimated that incidence should be high during the summer months. Soh et al. (1961) found only 2.5-5.2% cyst positive cases during May to August, while 5.2-6.1% during September to December. Clinical cases, however, are more frequent during warmer months. Sato (1913) analyzed amebic dysentery cases admitted to government hospitals and found that 10 out of 24 cases occurred in July and August.

Usually, more amebic patients are recorded during the warm months. An abundance of insects, availability of raw food, and weakened physical conditions due to hot temperatures may accelerate the higher incidence.

**Environmental factors:**

In many parts of Korea, people still utilize human excreta as fertilizer for growing vegetables. Since sanitary sewage and nightsoil treatment systems have not yet been provided in rural areas and many of the urban cities, the
high prevalence of amebiasis in the soil is recognized as a matter of course. Soh et al. (1959) detected *E. histolytica* cysts from 10 roadside soil samples even in Seoul. Infected food handlers may play an important role in the transmission of the protozoa. Flies, cockroaches, and other coprophagous may also contaminate food and drinking water.

A high incidence of amebiasis is found in areas where pollution is poorly controlled. Brooke et al. (1956) found 87% of *E. histolytica* carriers in a prisoner-of-war camp on Koje Island. Kim (1967) made an extensive study of Jeju Island where the inhabitants fed hogs with human feces using the pigsties as toilets. Thus hog feces and manure heaps may act as the spreading sources of the protozoa. It is noteworthy that drinking water which was kept in jars contained cysts in 3 out of 76 samples examined. In the same community, two direct fecal examinations revealed 24.4% positive rate for *E. histolytica* cysts out of 865 samples. Almost 49.0% of the positive cases complained of liver enlargement and abdominal discomfort. The high rate of cyst carriers may induce dysentery followed by complications such as hepatic amebiasis and some other forms of extra-intestinal amebiasis.

**Group living:** Brooke et al. (1956) reported 87% to be *E. histolytica* carriers in a prisoner-of-war camp on Koje Island where sanitary conditions were poor. Cho and Soh (1970) also reported 32.4% positives among 105 children in an orphanage in Gaejong, Jeonra-buk-Do, but among the 149 inhabitants of the surrounding villages only 7.4% were positive. This reinforces the theory that poor sanitation or crowding is directly related to the horizontal spread of the infection.

**Family infection:** In households where sanitation is poorly practiced, family infection cannot be overlooked. Cho et al. (1967) examined *E. histolytica* cyst carriers in households on Jeju-Do. Of 65 households, 51 (78.5%) were positive. Among them, only 12 households (23.5%) had single positive case among their families, and 39 (76.5%) had 2–5 persons infected in the same family. Kim (1971) visited 62 houses in which infants were positive for *E. histolytica* infection. Most of the families were low-income and undernourished, and the drinking water and food were handled in a poor manner. By examining the respective family of the infants, the protozoa positives were 46.2% among mothers, 34.1% among fathers, and 38.3% among other family members. *E. histolytica* cyst positives were mothers 17.3%, fathers 7.4%, and siblings 9.0%. The figures suggest that mother may play an important role as the source of amebic infection among families. This fact again emphasizes that family infection is likely to be common in poorly controlled environments.

**RESEARCH ON ENTAMOEBA HISTOLYTICA IN KOREA**

Amoebae belongs to the order Amoebina of the phylum Protozoa. The order Amoebina is characterized by having endoplasm and most of its species are cyst producers. Reproduction is exclusively asexual, usually by binary fission. They inhabit fresh or salty water in a free-living state, but some of them are parasitic to human beings and animals as endoza. The order has families: Naegleriidae, Amoebidae, Endamoebidae and Paramoebidae. Within the families, some of the species which belong to the former 3 families have been recognized as human parasites. Among the families, *Entamoeba histolytica*, *E. coli*, *E. gingivalis*, *Iodamoeba butschlii*, *Endolimax nana* and *Dientamoeba fragilis* of Endamoebidae were recorded as parasites of man in Korea. With regard to pathogenicity, only *E. histolytica* has been regarded as producing clinical symptoms, even though *Dientamoeba fragilis* was reported as pathogenic potential (Yang and Schalten, 1977). For this reason, contents of the following review focuses on *Entamoeba histolytica* and its infection.

**Outline:**

The first description about *E. histolytica* was by Moriyasu (1913) entitling “A case of liver abscess complicated by *Entamoeba histolytica*
Table 2. Analysis of publications about E. histolytica or amoebiasis by contents of the researches in Korea, 1913~1990

<table>
<thead>
<tr>
<th>Period</th>
<th>Year</th>
<th>No. publications</th>
<th>Clinical (%)</th>
<th>Basic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1913~1945</td>
<td>77</td>
<td>23(29)</td>
<td>54(71)</td>
</tr>
<tr>
<td>2</td>
<td>1945~1990</td>
<td>109</td>
<td>47(42)</td>
<td>62(58)</td>
</tr>
<tr>
<td>Sum</td>
<td></td>
<td>186</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Clinical: case report, treatment, diagnosis
Basic: epidemiology, pathology and in vitro or in vivo experiment

infection”. Since then 186 papers have recorded so far on literatures. For convenience sake, the period shall be divided into 2 phases. First half was from 1913 to 1945 (period of Japanese imperialism) and the second half has been from 1945 to 1990 (Table 2).

Factors influencing the pathogenicity of E. histolytica:

Factors which associate with pathogenicity of E. histolytica infection are still disputed. Aiming to clarify some of them, Soh and his associates performed several experiments with strains isolated in Korea (Table 3)

Table 3. E. histolytica isolated in Korea

<table>
<thead>
<tr>
<th>Source</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyst from carrier stool</td>
<td>YS-1, 3, 5, 9, 14, 15, 16, KY-15, 40, 41, 43, 44, 45, 48, 49, 52-57, 59, 60</td>
</tr>
<tr>
<td>Trophozoite from dysentery stool</td>
<td>YS-10</td>
</tr>
<tr>
<td>Cyst in stool from liver abscess case</td>
<td>YS-12, KY-9</td>
</tr>
<tr>
<td>Trophozoite from liver abscess</td>
<td>YS-23, 24, 25, 27 KY-9, 27, 37</td>
</tr>
</tbody>
</table>

The rat was the principal laboratory animal, although mouse, rabbit and golden hamster were also utilized when necessary. HK-9 and NAMR-2 were used as reference strains.

(1) Cultivation: Tanabe and Chiba (1928) tried a diphasic medium for cultivation of E. histolytica. The liquid phase was rabbit serum mixed with Ringer’s solution. Since then a number of modified media were devised and described as new or improved methods in Korea.

However, no device was satisfactory for continuous growth and propagation of the amoebae. In the meantime, Cho (1968) tested sera of rabbit, dog, pig, human, cow, and calf, and found that propagation of amebae was most luxuriant in a medium to which calf serum (2 drops in 15 ml) was added. He concluded that calf serum was excellent for culture of E. histolytica, providing a richer harvest of the parasite in a less amount of serum.

(2) Strains: To clarify pathogenicity in relation to size, Lim et al. (1965) analyzed cysts from 59 fecal samples of chronic amebiasis cases and compared the clinical signs with the sizes of the cysts, but found no evidence that the large race (9.0~10 μm) only was responsible for amebic colitis and the small race (6.1~7.2 μm) was not a tissue invader. However, there are still considerable disputes whether the small race E. histolytica is as invasive as the large race.

Ignoring size differences, Soh et al. (1969) studied the virulence of the strains collected in the laboratory (Table 3).

1) YS-9 strain: Isolated in September 1966 from the feces of a 51-year-old male with liver abscess
2) YS-14 strain: Isolated in January 1967 from a 63-year-old healthy cyst-passers stool
3) YS-15 strain: Isolated in January 1968 from feces of a 51-year-old symptomatic cyst carrier
4) YS-16 strain: Isolated in February 1968 from a healthy cyst passing 45-year-old female
5) YS-24 strain: Isolated in June 1969 from the liver abscess of a 33-year-old male in Severance Hospital
6) YS-25 strain: Isolated in June 1969 from the liver abscess of a 42-year-old male
7) NAMRU-II strain: The strain was isolated in 1967 from an acute dysentery patient (Vietnam inhabitant) by rectoscopic method and shared with the courtesy of Professor J.H. Cross, US-NAMRU-2 (Taiwan). E. histolytica were inoculated intracecellary to Sprague-Dawley strain or hybrid rats and determine the pathogenicity by Neal’s score method (1968). It was used as
a reference strain for the Korean strains.

The criteria for scoring were:

<table>
<thead>
<tr>
<th>Contents</th>
<th>Score</th>
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<tbody>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Slightly less solid than normal</td>
<td>1</td>
</tr>
<tr>
<td>Slightly mucoid</td>
<td>2</td>
</tr>
<tr>
<td>Mucoid, some solid matter present</td>
<td>3</td>
</tr>
<tr>
<td>No Solid matter, white or yellow mucus only</td>
<td>4</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Wall</th>
<th></th>
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<tbody>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Slight thickening</td>
<td>1</td>
</tr>
<tr>
<td>Marked local thickening and contraction</td>
<td>2</td>
</tr>
<tr>
<td>Extensive thickening and contraction</td>
<td>3</td>
</tr>
<tr>
<td>Cecum shapeless, extensive ulceration with abscess formation</td>
<td>4</td>
</tr>
</tbody>
</table>

The scores were 5.0~7.8 in the 30-day-old rats, 4.2 in the 40-day-old ones, 2.5 in the 60-day-old ones, and 2.8 in the 90-day-old ones. NAMRU-II strain showed similar invasiveness according to the host's age. The results indicate that establishment of pathological change due to amebic infection may depend upon host factors such as age and nutrition.

Virulence of the strains was examined in the weaned Sprague-Dawley rats. Each experimental group was composed of rats with the same mother. Each animal was inoculated intracecally with 100,000 amebic organisms belonging to strains YS-14, YS-15 and YS-16 which originated from cyst carriers, and YS-24 and YS-25 strain amebae isolated from liver abscess. Average cecal scores were 1.0 for the YS-14 strain inoculated group; 3.0 for the YS-15 strain group; 2.2 for the YS-16 strain group; 3.0 for the YS-24 strain group, and 6.3 for the YS-25 strain group. Comparing those results with the YS-9 strain and NAMRU-II strain amebae, the YS-14 and YS-16 strains showed no virulence, the YS-15 and YS-24 strains were moderately invasive, and only the YS-25 strain was determined to be highly invasive strain. The findings suggest that the strains which originated from nonclinical amebiasis are more invasive than the strains from cyst-passers in general, but the YS-14 strain which had the score of 1.0 showed marked increase to 5.5 at the second animal passage. It would suggest that E. histolytica itself is essentially pathogenic, but likely to be changeable by conditions such as artificial culture, host resistance and some other unidentified factors.

(3) Temperature adaptation: Several reports indicate that temperature adaptation is related to the virulence of the strain, though the results were diverse. Cabrera (1958) reported that the strain which adapted to lower temperature showed higher virulence. On the contrary, Neal and Johnson (1968) observed the opposite. They found that 5 strains of E. histolytica adapted to room temperature and propagated normally but showed lower infectivity and produced no cecal ulceration experimentally. To clarify the foregoing discrepancies, Cho et al. (1972) undertook a study of the relationship between temperature adaptation and pathogenicity. The overall results suggested that the lowest critical temperature of these strains was 30°C, and survival time of the strains did not always correlate to temperature conditions.

Under three different temperatures; 37°C, 32°C, and 30°C, all strains originated from noninvasive cysts, one highly invasive trophozoite strain (YS-23) showed the highest growth peak at 32°C, while 3 strains originating from highly invasive trophozoites showed the highest peak at 37°C, but poor growth was observed at 30°C in all strains. The results suggested that strains originating from nonclinical cases were more adaptable to lower temperatures, whereas strains from pathological lesions were more propagative at body temperature of 37°C.

(4) Red blood cells and hemolysis: There have been disputes on the role of red blood cells for propagation of E. histolytica. Lösch viewed it (1875) as nutrient, but Schaefer and Iralu (1961) as toxin. Craig (1927) found that E. histolytica had hemolytic ability, and Schaefer and Iralu (1963 & 1967) reported the ability differed according to the strain of E. histolytica. To clarify the confusion, Ro (1967) tried a study on the role of red blood cells. The strains sampled were the YS-1, 5 and 9 strains from
cyst carriers and the YS-10 strain from an acute
dysentery case. In the study, hemolytic ability
differed by the strain of *E. histolytica* and
animal. The YS-1 strain lysed the red cells of
pig, sheep and ox, but not of rabbit, dog and
man. YS-5 strain lysed the red cells of ox, but
not of rabbit, dog, pig, sheep and man. The
YS-9 strain lysed red cells of sheep, but not
other, and the YS-10 strain lysed the red cells
dog and sheep slightly, but not others. In
this way, each strain showed selective ability
to hemolyse red blood cells of different animals.

Hemolysis was considered to play an important
role in propagation of the ameba in medium.
Ro (1967) added 0.2 ml of 0.5% rabbit or sheep
red blood cell suspension to the media. In the
experiments he found that rabbit red cells were
hemolysed within 4 days, whereas sheep cells
took 2 days. The growth of the amebae was
checked every 2 days for a total of 10 days.
To the rabbit cells, 5,800 amebae were added.
On the second day, the numbers were 1,450/ml
in the red cell added media but 19,600/ml in
the non-added control. On the fourth day, only
100/ml were found in the former but 13,700 in
the latter. But after the hemolysis occurred, the
yields reversed. Similar results were observed in
the medium to which sheep cells had been added.
The number of the amebae showed a lag phase
until completion of hemolysis, then increased
overwhelmingly in the control group. Thus, it
is conjectured that normal red blood cells have
some inhibitory action on the growth of *Ent-
amoeba histolytica*. Nevertheless, once they are
hemolysed, the components may contribute as
nutrients to the growth.

(5) **Enzymes:** Pathogenicity of *E. histolytica*
may rely on enzyme activities. To elucidate the
theory, Cho et al. (1973) conducted a combined
cytochemical and electron microscopic study in
order to demonstrate acid phosphatase activities
in trophozoites of *E. histolytica*.

In the study, *Entamoeba gingivalis*, a com-
mensal in the buccal cavity, was utilized as the
control.

In the cytoplasm of both amoebae, various
reaction precipitates were observed in the vacu-
ole-limiting membrane, the vacuole membrane
and its contents, and in lysosome-like structures.
However, in *E. histolytica*, the reaction products
were distributed evenly over the entire surface
of the plasma membrane, suggesting that surface
enzymes may play a role in the invasiveness of
*E. histolytica*. *E. gingivalis*, however, showed
no activity of acid phosphatase on the plasma
membrane, except in the portion of the uroid-like
structure.

In recent years, Sargeaunt et al. (1978 & 1980)
emphasized that the different electrophoretic iso-
enzyme pattern of *E. histolytica* might contrib-
ute to distinguishing the pathogenic strain from
the non-pathogenic. To confirm the theory, Soh
et al. (1984) performed a follow-up study with
25 strains.

The samples were from amoebic colitis pa-
tients, amoebic liver abscess cases, or asymptomatic
cyst passers from various parts of Korea and
cultured for isoenzyme studies.

Starch-gel electrophoresis for phosphoglucose
isomerase (E.C. 5,3,1,9; PGI) phosphogluco-
mutase (E.C. 2,7,5,1; PGM), malic enzyme
(E.C. 1,1,1,40; ME) and hexokinase (E.C.
27,7,1,1; HK) was utilized by Maazoun’s
technique. To elucidate the relation of the elec-
trrophoretic patterns of isoenzymes and clinical
episodes, rats were inoculated with each of the
amoebae strains in the cecal region, and then
sacrificed one week after inoculation for obser-
vation of pathological changes on the cecal walls.

The electrophoretic patterns of the 25 strains
were grouped into 12 types. All strains showed
a single ME band in the same position which was
classified as characteristic of *E. histolytica*. HK revealed
4 bands which migrated relatively faster, but
none were decisive enough to define a clinical
association of the respective strain. GPI showed
2 bands, but neither band denoted a pathogenic
association. The single band in PGM appeared
in 3 pathogenic strains, but 2 strains from simple
cyst passers showed a similar pattern. Intracel-
lar inoculation of the strains from clinical cases did
not produce any noticeable pathological changes
in the caecum of rats, even though the infectivity was high. As far as the results indicated, no clearcut relationship between the zymodeme pattern and pathogenicity was recognized.

(6) Mast cells: In establishment of clinical amoebiasis, host factors are considered to have a similar level of biological characteristics as *E. histolytica*. Soh and his associates (1981) carried out some studies on host factors which may involve enhancing the pathogenicity of *E. histolytica*.

Mast cells may be related to allergic states in the course of *E. histolytica* infection, as Im *et al.* (1975) suggested after experimental studies. Mice weighing about 16 gm were used for experimental groups of sham and experimental infection. The sham group was injected intracereally with 9 liquid medium only, but 5,000–50,000 amebae containing liquid medium was injected in the experimental group. Mast cells in the mesenterium and eosinophils in peripheral blood were examined from the first day. Mesenteric tissues from the region of the terminal ileum were fixed in methyl alcohol and stained with Pugh's solution. On the seventh day ulcers were found in the cecal walls in all the mice inoculated with the amebae. The number of mast cells in the mesenteric tissues of the infected group increased from the first day of the infection and persisted up to the 34th day of the observation period. Degranulation and disruption of the mast cells increased in the infected group in comparison with sham operation group and the control, but no difference was discerned by strain of *E. histolytica*. Blood eosinophilia was also noticed in the infected group and persisted during the entire observation period. Thus, it was confirmed that *E. histolytica* infection may contribute to the degranulation of mast cells provoking an increase of the eosinophils. It may be the secondary reaction due to degranulation of mast cells.

(7) Nutrition: Diet may correlate with the intensity of *E. histolytica* infection (Sadun *et al.*, 1952), but the relationship between the nutritional condition of the host and the infectivity of *E. histolytica* was not clearly defined. Choi (1969) performed an experimental study on the susceptibility or resistance to *E. histolytica* infection using various levels of dietary proteins. Young rats of both sexes were used for the study and were divided by diet into 4 groups

1) Group D: Protein depleted diet (rice power 85% without casein)

2) Group L: Low protein diet (rice power 80%, casein 5%)

3) Group M: Moderate protein diet (rice power 70%, casein 15%)

4) Group H: High protein diet (rice power 60%, casein 25%)

Olive oil (4%), an inorganic salt mixture (4%), cod liver oil (2%) and yeast (5%) were added equally to each diet.

*E. histolytica* was inoculated intracereally to rats which had been maintained on the above formulated diets for 15 to 17 days and sacrificed 14 days after inoculation.

The number and size of the crater-like ulcers in the ileocecal area were measured under stereomicroscope, and then histo-pathological studies were carried out. The ulcers were divided according to the severity of the ulceration;

Degree I: one pin point ulcer in the ileocecal area

Degree II: one or two ulcers, 1–2 mm in diameter

Degree III: more than two ulcers.

The growth of the protein-depleted diet group, group D, was more markedly reduced in body weight than that of any other groups from the 3rd day of the diet. The amount of ingested protein did not show any difference by group statistically. The average amount of diet consumed was 30–40 gm per day per individual rat. Amebae were found in the contents of the ileocecal area of the rats: 100% in the group D, 85.7% in the group L, 73.6% in group M and 44.4% in the group H. Generally the gamma globulin level increased in all groups after inoculation with the amebae, especially in the hyperprotein diet group, and the value of the total serum protein in the group D (6.87 gm%)
was the lowest among the groups.

Histologically, the percentage of ulceration was 60.0% in the group D and 21.4% in the group L. In the groups M and H, there was only one case showing cecal ulceration. The cecal ulceration rate and infectivity increased in the low-protein diet group in compared to the groups fed a high protein diet. According to the results it was concluded that low protein diet retarded the growth of the host and reduced the resistance of the host to amebic infection.

(8) Hormones: Sexual hormones and some corticosteroid hormones may influence the infectivity, intensity and pathogenicity of *E. histolytica*. However, there have been diverse views regarding this dismerit.

Teodorovic et al. (1963) presented some evidence for an adrenal effect. He observed a marked exacerbation of *E. histolytica* pathogenicity in mice treated with corticosteroids. Conversely, Villarejos (1962) reported that cortisone did not increase the susceptibility of rats to amebic infection. Solomon (1964) reported that testosterone promoted the susceptibility of the gonadectomized animal to parasitic infection in comparison with the effect of ergosterone. Lee (1968) attempted a study to elucidate whether physical, sex hormonal, or toxic bacterial stimulation affected intestinal infection with *E. histolytica*.

The YS-9 strain isolated from a dysentery case was used. The rats were castrated or oophorectomized. Animals, sham-operation or operation but without hormones, were used as the controls. As sex hormones, testosterone and ergosterone were used. Testosterone propionate, 50 mg/ml, was injected intramuscularly 4 times every other day before and after inoculation with 150,000 organisms. Estradiol benzoate, 2 mg/ml, was used as a female hormone 4 times every other day before and after inoculation with 200,000 organisms.

In the testosterone-treated cases, 10 and more ulcers were observed in 4 out of 13 rats. Next was the castrated control group, and the least number of ulcers was in the normal control group. The average number of ulcerations per rat was 9 in the testosterone treated group, 4 in the castrated group, and none in the normal control group. Ulcers above 2 mm in diameter were found predominantly in 45.5% of the experimental animals. However, no appreciable effect was observed on infectivity and pathogenicity in the ergosterone-treated group. Previous infection with enteric bacteria also enhanced pathogenicity.

Lee (1968) observed the effect of previous infection of *Shigella dysenteriae* in rats on amebic infection. When rats infected orally with a 1.0 ml of *S. dysenteriae* suspension one week previously, and inoculated with 300,000 amebae intracereally, the susceptibility to *E. histolytica* was strongly enhanced. The pathological changes in these rats were more numerous than in the mechanical stress-only group and in the merely infected group. The average number of ulcerations per rat was 9, whereas it was 7 in the stress-only rats and 3 in the infection-only rats.

Influence of steroid hormones upon the production of hepatic abscess by *E. histolytica* was investigated by Lee et al. (1989). Golden hamsters were utilized for the experiment. Dexamethasone, methyl prednisolone and hydrocortisone were injected intraperitoneally to golden hamsters every 3 days for a period of up to 2 months. After then *E. histolytica*, 10,000/0.1 ml, was inoculated into the liver.

One week (7~8 days) after the infection, the hamsters were sacrificed, and examined pathologically and parasitologically. In general, the steroid groups produced more number and larger size of abscesses than the controls. From pathological and microscopical viewpoints, the grade was different by groups of dexamethasone, methyl prednisolone, hydrocortisone, and control in decreasing order even though no significance was proved. Histologically, neutrophil and fibroblast infiltrations were noticed bordering the abscesses together with *E. histolytica*.

In general golden hamsters which were inoculated with steroid hormones, especially dexamethasone, were susceptible to infection of *E. histolytica*, suggesting a phenomenon of
(9) **Stress**: The effects of some stresses such as shaking or compression upon the intensity of *E. histolytica* infection was studied by Lee (1968).

All rats, except for those of the normal control group, were shaken before their inoculation with amebae. They were put into a cage on the shaking machine (Arthur H. Thomas Co.), and stressed 4 hours daily for a week. Rats in the shaking stress group showed more severe pathological changes than in the control group of non-shaking stress.

In another experiment, the exposed ceca of laparotomized rats were compressed with surgical forceps to produce congestion, then inoculated intraceally with 300,000 parasites. The direct physical damage to the ileocecum also showed enhancement of infectivity. Thus, Lee (1968) found that stress or physical damage caused more pathological changes.

**Hepatotoxic agents and amebic liver abscess:**

Amebae might enter the liver by various routes—blood and lymph vessels or directly from the adjacent portions of the colon—although the portal vein is the most probable route. Amebae reach the lumina of the large branches of the portal vein, then the smaller ramification of the veins and the adjacent sinusoids of the hepatic lobules. On the next step, mechanical and chemical damages are encountered as predisposing factors for formation of amebic abscess. Hepatic injury by migrating larvae of *Toxocara canis* (Krupp, 1956) or introduction of insoluble matter such as glass particles into liver tissues (Gogler and Knight, 1974) may provide a precondition for abscess formation by the invading amebae. Chemical damage also provides a favorable condition for their establishment. Roh and Kim (1948) found that 83.7% of the 159 abscess cases were identified as heavy drinkers or habitual alcoholics. Based on such previous reports, Im and Kim (1976) succeeded in establishing amebic liver abscess experimentally by giving hepatotoxic materials before infecting with amebae. Thioacetamide and carbon tetrachloride were used as hepatotoxic agents.

Four per cent thioacetamide was injected intramuscularly in the thigh at a dose of 0.25 mg/100 gm of body weight, and the amebae were inoculated 3 hours later. Ten per cent carbon tetrachloride in liquid paraffin was introduced to the stomach via nasogastric tube, at a dose of 0.1 mg/100 gm of body weight, and amebae were injected 24 hours later. Six to 11 days after the inoculation, the animals were sacrificed and examined for parasitological and pathological findings. Separately, thioacetamide and carbon tetrachloride were given to healthy rats in the same amounts as above, and the excised liver examined 3 hours and 24 hours after their administration for any pathological change of the organ.

They found that chemical hepatic injuries fostered the formation of amoebic liver abscess even in less susceptible animals such as rat.

**DIAGNOSIS**

Unless the symptoms and signs are pathognomonic, accurate diagnosis requires demonstration of *Entamoeba histolytica* in its trophic or cystic stage. In case the pathogenic agent cannot be isolated, scanning or ultrasonograph are the helpful methods and immunologic diagnosis is an alternative and promising method for this purpose, particularly in extra-intestinal amebiasis.

Immunodiagnostic methods are complement fixing antibody test (CFT), ameba immobilization test (AI), indirect fluorescent antibody test (IFA), soluble antigen fluorescent antibody test (SAFA), gel-diffusion precipitation test, immunoelectrophoresis (IEF), indirect hemagglutination test (IHA), intradermal test, bentonite flocculation test, latex agglutination test, and the ELISA technic.

But from the viewpoint of reproducibility, each test has its own merits and demerits. As a routine procedure in the laboratory, however, IHA, IFA, and AI are commonly used because the actual assessment time requires only 1 to 3 hours compared to CFT and precipitation test
which need a longer time (Soh, 1981a).

In order to find a more reliable and reproducible way to diagnose the infection, Min (1975) conducted comparison studies with some commonly accepted immunological tests, and drew the conclusion that a combination method with IHA, IFA and AI was the most reproducible one to attain the reliable method, but only positive test or concomitant negativity of the 3 tests were clinically insignificant, and 2 tests positive may suggest further examination for decisive diagnosis.

TREATMENT

Continuing from the “Emetine” era up to 1950, nitroimidazole derivatives were first introduced in the 1960s in Korea as a form of metronidazole. Based on its high effectiveness by in vitro and in vivo tests (Cho et al., 1969), a number of clinical trials confirmed that various forms of nitroimidazole compound were the drug of choice in treatment of intestinal and extraintestinal amoebiasis (Cho et al., 1976; Soh et al., 1978).

In the treatment of hepatic amoebic abscess, open drainage with an antiamoebic drug was the routine method until Ludlow (1924 & 1926) developed an aspiration method during his service as Professor of Surgery in Severance Hospital in Seoul, which became an epoch-making method of reducing the complications of the open drainage method. Since nitroimidazole compound was introduced as the most effective chemical for amoebiasis treatment, emetine was replaced with the compound, and it is the most recommendable way for the treatment of any kind of amoebiasis (Soh et al., 1978).

Indirect haemagglutination antibody test (IHA) or complement fixation antibody test (CF) are currently used for diagnosis of Entamoeba histolytica infection, but none of them are conclusive, so that a combination method is usually applied. To eliminate such complexity and time consumption, biotechnical method by a genetic method would be desirable.

EPILOGUE AND PROSPECTS

In summarizing the results of the experimental studies up to the present, it is conjectured that the pathogenicity of Entamoeba histolytica or establishment of amoebiasis is not unique but differs by strain. A nonvirulent strain is more likely to adapt to lower temperature down to 32°C. This is not so in the strains which originate from clinical cases. Iso-enzyme patterns may roughly characterize pathogenic strains from nonpathogenic. Red blood cells may contribute as nutrients for growth of Entamoeba histolytica only after they have been hemolyzed, and they are toxic to the amebae as long as they remain intact. A low protein diet and stress may facilitate the establishment of amoebiasis; male sex hormones or previous infection by enteric bacteria provide a more advantageous condition than the female hormone. Hepatotoxic agents will accelerate amoebic hepatitis.

Desirable research in the future be focused in the following directions:

1. Distinguish the virulent strain from the nonvirulent. Roughly 6% of the population are simple cyst carriers, but there is no way to distinguish them. Clarification could contribute in reserving unnecessary treatment and establishing more effective control measures.

2. Development of diagnostic method utilizing DNA probe.

REFERENCES


