Update
Le point

Articles in the ***Update*** *series give a concise, authoritative, and up-to-date survey of the present position in the selected fields, and, over a period of years, will cover many different aspects of the biomedical sciences and public health. Most of the articles will be written, by invitation, by acknowledged experts on the subject.*

Les articles de la rubrique ***Le point*** *fournissent un bilan concis et fiable de la situation actuelle dans le domaine considéré. Des experts couvriront ainsi successivement de nombreux aspects des sciences biomédicales et de la santé publique. La plupart de ces articles auront donc été rédigés sur demande par les spécialistes les plus autorisés.*

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Amoebiasis and its control*

A WHO MEETING[1]

On a global scale, 480 million people were recently estimated to be infected with Entamoeba histolytica *and some 48 million people suffer from invasive amoebiasis. The latter, in the intestinal and extra-intestinal forms, accounts for 40 000 to 110 000 deaths annually. In view of this considerable mortality and morbidity, more research is urgently needed so that more effective control strategies may be introduced.*

This Update article reviews the magnitude of the problem, clinical and laboratory diagnosis, present treatment and prevention measures, possible control strategies, and research priorities.

Amoebiasis is an infection of humans with the protozoan *Entamoeba histolytica* and has a worldwide distribution (*1*). The pathogenic effects of this infection and the varying clinical expressions depend on many host and parasite factors and their dynamic interplay. Thus it is difficult to draw a clear-cut distinction between infection and disease.

Amoebiasis as a disease is caused by potentially pathogenic strains of *E. histolytica*, the characteristics of which are (i) a higher rate of erythrophagocytosis; (ii) a greater propensity to agglutinate with the lectin concavalin A, reflecting a higher number of exposed glucose- and mannose-containing receptors, and inhibition by a characteristic *N*-acetyl-D-glactosamine (GALNAc) of amoebic binding to target cells via a soluble lectin (*2*); (iii) a lack of overall surface charge, which may facilitate interaction with the negatively charged mammalian cells; (iv) a more potent cytopathic effect *in vitro*; (v) an ability to grow in axenic media; (vi) the production of lesions in experimental animals (*3*); and (vii) a typical isoenzyme pattern.

Isoenzyme electrophoretic mobility analyses performed on amoebae isolated from stool samples from four continents have so far identified 7 potentially pathogenic and 11 non-pathogenic zymodemes (*4*).

Major host factors contributing to the unpredictability of amoebic infections in man are (i) the physicochemical environment in the gut under the influence of, for example, the

* This article, which was prepared by Dr A. Davis and Dr Z. S. Pawlowski, Parasitic Diseases Programme, WHO, Geneva, Switzerland, is based on the report of a WHO meeting on strategies for control of amoebiasis. The meeting was held in Geneva on 6-8 February 1984. Requests for reprints of this article should be sent to the Parasitic Diseases Programme, World Health Organization, 1211 Geneva 27, Switzerland. A French translation of this article will appear in a later issue of the *Bulletin*.

[1] A list of the participants in the meeting is given on page 425.

bacterial flora, mucus secretions and intestinal motility, and (ii) the degree of immunological resistance (specific antiamoebic antibodies are produced when tissue invasion occurs; immune serum produces rapid lysis of *E. histolytica* trophozoites *in vitro*; accumulated experimental evidence strongly suggests that cellular immunity plays an important part in controlling the recurrence of invasive amoebiasis (*5*)).

The term "invasive amoebiasis" reflects both parasite and host factors which result in pathological lesions. Invasive intestinal amoebiasis is characterized by the following markers: (i) clinical symptoms and signs of disease; (ii) presence of haematophagous trophozoites in the stool or rectal scrapings; (iii) characteristic changes in the intestinal mucosa at endoscopic examination; (iv) positive serological tests for specific antibodies. Many gradations of pathological change and clinical expression occur in invasive amoebiasis.

The existence of chronic intestinal amoebiasis as a clinical entity, characterized by a prolonged course, a tendency to recur, non-specific bowel or general symptoms and associated cellular infiltration of the intestinal mucosa, still requires confirmation.

REVIEW OF CURRENT INFORMATION

Magnitude of the problem

The distribution of luminal infection with the parasite, as measured by the presence of cysts in stools, is worldwide. These infections are asymptomatic and may affect from less than 5% to over 50% of any given population; it was estimated that, in 1981, 480 million people carried *E. histolytica* in their intestinal tract (*6*).

Only a small percentage of those having intestinal infection will develop invasive amoebiasis, the main forms being dysentery and liver abscess. Serological surveys for antibodies, which are used to measure the proportion of the population with past or current invasive disease, suggest that approximately one-tenth of the total number of infected people, i.e., some 48 million people annually (*6*), have intestinal mucosal or liver invasion; amoebic dysentery occurs five to 50 times more frequently than liver abscess.

Amoebiasis causes death mainly when it manifests itself as liver abscess or fulminating colitis; 2–10% of persons with liver abscess may die whereas the mortality among those with fulminating colitis is almost 70%. It is probable that invasive amoebiasis accounts annually for 40 000 to 110 000 deaths in the world. Thus, amoebiasis occupies an important place on the list of parasitic causes of death on a global scale.

Amoebiasis is more closely related to sanitation and socioeconomic status than to climate. It is known to be a major health problem in China, Mexico, the eastern portion of South America, south-east and west Africa, and the whole of south-east Asia including the Indian subcontinent, but reliable information is available from only a few countries.

In addition to being a potentially lethal disease, invasive amoebiasis has important social and economic consequences. Temporarily incapacitating infections, which are frequent in adult males in the wage-earning age group, may require several weeks of hospitalization and 2–3 months for full recovery. Amoebiasis may cause clinical problems in persons with immunodeficiency, homosexuals, immigrants from certain tropical countries, and travellers.

In view of the substantial morbidity and mortality caused by *E. histolytica*, more research into better methods of diagnosis, treatment, and prevention is clearly necessary and an improvement of control strategies is essential.

It is hoped that the impact of the International Drinking Water Supply and Sanitation Decade (1981–90) will reduce the magnitude of the problem, but adverse factors such as

population growth, uncontrolled urbanization, and economic crises may militate against success in control.

Clinical forms of amoebiasis

In countries where amoebiasis is an important health problem, the majority, approximately 90%, of individuals with colonic *E. histolytica* infections are carriers, while the remainder have invasive intestinal amoebiasis (*7*). The latter condition is usually characterized clinically either by acute amoebic dysentery with bloody, mucous stools, colicky pain, and rectal tenesmus, or by intermittent diarrhoea, frequently with blood-stained faeces. In general, there is no fever or other systemic manifestation and the symptoms disappear after a few days of treatment or even spontaneously.

The following three forms of clinically severe intestinal amoebiasis—fulminating amoebic colitis, amoeboma of the colon, and amoebic appendicitis—are common, and occur more in adults than in children.

Fulminating amoebic colitis is characterized by the passage of numerous bloody stools, generalized abdominal discomfort, colicky pains preceding evacuation, and rectal tenesmus which tends to be constant and intense. General manifestations include fever, dehydration, and rapidly progressive prostration. Advanced lesions frequently produce intestinal haemorrhages, or perforation followed by peritonitis (*3*). Specific antiamoebic treatment may be ineffective and surgery may be required.

Amoebomas are pseudotumoral lesions that occur predominantly in the vertical regions of the colon, the caecum, and the rectum and are associated with vague abdominal symptoms, occasional bloody diarrhoea, and a palpable mass. They respond well to specific treatment.

The clinical manifestations of amoebic appendicitis are similar to those of bacterial appendicitis. Diarrhoea with blood-stained stools is a frequent accompaniment and a distinguishing feature.

The most common extra-intestinal form of invasive amoebiasis is amoebic liver abscess. Amoebic abscesses are ten times more common in adults than in children, with a higher frequency in males (3:1). Only about 9% of patients may have an associated amoebic recto-colitis. The clinical features of liver abscess, which may be of abrupt onset, are pain and tenderness in the region of the liver, wasting, and fever associated with chills and profuse night sweats. The less common extra-intestinal forms of invasive amoebiasis are pulmonary or cerebral abscess and skin amoebiasis.

Clinical and laboratory diagnosis

In the great majority of cases of invasive colonic amoebiasis, rectosigmoidoscopy and immediate microscopic examination of rectal smears and/or fresh stool samples for the presence of motile haematophagous trophozoites of *E. histolytica* are the most reliable diagnostic procedures. In these cases, differentiation of amoebae by characteristics of nuclear structure is rarely necessary. The presence of Charcot-Leyden crystals and absence of pus cells in the stool may be very significant for amoebiasis and helpful in the differential diagnosis with shigellosis. Because of the amoebic cytolethal effect, pyknotic or disrupted leukocytes are characteristically seen in the stool (*8*). A good quality microscope is needed, as well as adequate training of the laboratory personnel. Merthiolate-iodine-formaldehyde (MIF), polyvinyl alcohol (PVA), and sodium acetate formalin (SAF) can be used for the fixation and transportation of stools, whenever confirmation of the diagnosis in a reference centre is required. Serological tests for antiamoebic antibodies are positive in approximately 75% of cases of colonic invasive amoebiasis. X-ray examination is particularly helpful in fulminating colitis, amoeboma, or peritonitis.

In the majority of cases of amoebic liver abscess, in addition to the clinical presentation, a polymorphonuclear leukocytosis is present. Antiamoebic antibodies can be detected in the serum of 95% of patients with amoebic liver abscess. X-ray examination shows elevation and hypomotility of the right hemidiaphragm. Scintillography, ultrasonography, and computerized axial tomography have greatly facilitated determination of the location and number of amoebic liver abscesses and evaluation of treatment.

Many serological tests for specific antibodies have been introduced as adjuncts to support the diagnosis of amoebiasis. The various tests currently employed include indirect haemagglutination (IHA), enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence (IIF), countercurrent-immunoelectrophoresis (CCE), and agar gel diffusion (AGD). These serological tests are particularly useful in the detection of extra-intestinal amoebiasis such as amoebic liver abscess, since stool examination is often negative for *E. histolytica* in such cases. All patients thought to have inflammatory gut disease should have a serological test for amoebiasis because of the potentially fatal consequences of giving steroids to patients with amoebic colitis and the uncertain quality of the parasitological stool examination in many laboratories. The tests are also useful as an epidemiological tool for the detection of invasive amoebiasis in a population. However, since positive titres may persist for months or years after successful treatment, it is difficult to differentiate between active and past infections (*5*).

The IHA test is useful for the detection of circulating specific antibodies in invasive amoebiasis—i.e., amoebic liver abscess and acute amoebic dysentery—and was also used to classify individuals as asymptomatic carriers, or symptomatic patients, requiring treatment. Certain cut-off titres have been suggested for the establishment of a diagnosis of amoebic liver abscess and intestinal amoebiasis. However, in highly endemic areas this test, if not confronted with the actual clinical condition, is of limited diagnostic importance, since a large number of persons may have a very high titre due to a past infection without the presence of any active amoebic disease. In an endemic area, all tests for the detection of antiamoebic antibody—e.g., AGD, CCE, immunofluorescent antibody (IFA), latex agglutination, and ELISA—suffer from the same limitation as the IHA test, and for that reason tests based on antigen detection need to be developed and improved.

An immune complex dissociation radio-immunoassay (RIA), as well as an ELISA, have been developed for circulating *E. histolytica* proteins. The technique is an improvement on the previously described Sandwich assay for the detection of *E. histolytica* antigen (*9*). For such RIA procedures it is essential to use an extensively purified antibody, raised against washed *E. histolytica* trophozoites. The critical feature is the need to deplete those antibodies against culture-medium-derived bovine-serum proteins, which cross-react with human proteins. In the case of the ELISA, instead of radio-labelling, peroxidase-conjugated rabbit anti-*E. histolytica* antibody has been used.

It is now possible to use antigen-detecting techniques in clinical studies since they do not have the disadvantage of the IHA and other serological tests for antibody detection which fail to distinguish between present and past infection. The RIA could thus be of particular value: (i) for confirmation of the clinical diagnosis of invasive amoebiasis; (ii) for study of the sequelae of *E. histolytica* infections in man; (iii) for use as a marker of disease activity; and (iv) for use by molecular biologists to study these parasites, particularly to screen for the expression of *E. histolytica* proteins in recombinant bacterial clones. If, in further studies in different endemic countries, the test is indeed found to be a reliable marker of disease activity and invasiveness, it could be used for epidemiological studies by selected workers or centres.

An ELISA test for the detection of *E. histolytica* antigen in stools has now been developed. The applicability of this test at different levels of the health services requires assessment and evaluation. If successful, this test may eventually replace microscopy for survey purposes.

Treatment

The drug of choice for the treatment of invasive amoebic disease is a nitroimidazole such as metronidazole, but the treatment is relatively expensive. Several alternative nitroimidazole preparations exist and clinical experience of their use is growing. Emetine and its synthetic derivative, dehydroemetine, despite their toxicity, are still indicated for patients who are either unable to take oral medicines or are critically ill. Closed drainage of a liver abscess may be necessary if the abscess cavity is large and there is no substantial improvement within 48 hours of starting chemotherapy. The variation in the pathology and clinical expression of amoebiasis in different parts of the world requires that the preferred treatment regimen should be based on local experience in both optimal chemotherapy and surgical interventions.

Although diloxanide furoate, diiodohydroxyquinoline, and paromomycin have been used as intraluminal antiamoebic drugs, there is no single-dose treatment available that can yet be advocated. In some countries the total consumption of antiamoebic drugs is high, but their use by the poorer segments of the population, where amoebiasis is most common, is limited because of their relatively high price.

Prevention and control measures

The basic approach to preventing amoebic infection is by improvement of living conditions and education in countries where invasive amoebiasis is prevalent. Specifically, methods of attack are aimed at (i) improved environmental sanitation including water supply and food safety, (ii) early detection and treatment of infections and/or disease, and (iii) health education (*10*).

E. histolytica cysts are remarkably resistant to chemical disinfectants, including chlorination, and can survive a wide range of pH values and osmotic pressures. They die rapidly if dried, heated (to about 55 °C), or frozen (*11*). Faecal-oral transmission via hands or food is very common. Therefore, sanitation and personal hygiene have priority in the prevention and control of amoebiasis. The availability of sufficient water for washing hands and food may be more important than the quality of the water alone.

As there is usually no direct relation in time and place between exposure and onset of disease, and epidemic situations are rare, health education on amoebiasis should form part of the general education programme for controlling infections transmitted by the faecal-oral route, which should be addressed to mothers, schoolchildren, and persons with influence in the community.

POSSIBLE CONTROL STRATEGIES

Control of amoebiasis will be achieved through national and international programmes for the improvement of sanitation, water supply, and food safety. Until such improvements become a reality in regions endemic for amoebiasis, some specific modifications or extensions of already existing programmes will need to be devised and implemented.

Improved excreta disposal, water supply, and food safety

The most effective preventive measures for amoebiasis are the safe disposal of human faeces coupled with the elementary sanitary practice of washing hands after defecation and before eating. The protection of water supplies against faecal contamination is also important because the cysts of *E. histolytica* may survive for several days or weeks in water. In areas endemic for amoebiasis, heating or filtration techniques are more effective than chemical treatment of water. Environmental measures should also include the protection

of food and drink from flies and cockroaches and the control of these insects, although their role in spreading amoebiasis may not be as important as in shigella infections.

Practices aimed at avoiding or minimizing the contamination of food are more important, and theoretically easier, than destroying or reducing the contaminants. Thus, carriers who pass cysts and are involved in handling food, whether at home, at street stalls, or in catering establishments, should be actively detected and treated since they are major transmitters of amoebiasis. Mechanisms to motivate healthy practices in food handlers must be developed and implemented. In all these efforts, food safety cannot be dissociated from ingrained sociocultural habits, which present one of the major obstacles to success.

The high prevalence rates of amoebiasis are not adequately recognized by public health authorities as an indicator of deficient sanitation and poor general development.

Health education

In the long term a great deal can be accomplished in the prevention of amoebiasis through education of the public as well as health personnel at all levels. In endemic areas health workers should be reminded of the problem constantly, receive training in the specific diagnostic and therapeutic procedures, and participate actively in preventive measures.

Elementary hygienic practices should be propagated and constantly reinforced in schools, health care units, and the home through periodic campaigns using the mass media. The existing educational mechanisms developed for the national diarrhoeal diseases control programme should be used, and additional information (e.g., related to the safety of food) should be addressed to the adult population. The long-ingrained cultural habit of purchasing and eating food from street vendors poses a constant threat in some countries, since they may be a major source of infection which is not easy to control, nor is the habit easy to discourage.

Case management

The management of amoebiasis will differ according to whether clinical cases or persons with asymptomatic infections are being dealt with. It will also vary at different levels of the health services infrastructure.

A community health worker is not able to do more than refer a clinically suspected case to the nearest health centre, physician or hospital. At the health centre level, symptomatic cases can be treated effectively with metronidazole or another nitroimidazole compound and the clinical response in 48 hours may confirm the suspected diagnosis. Suspected cases of amoebic liver abscess with pain and tenderness in the right hypochondrium, along with other symptoms, should be referred to the nearest hospital.

Hospitals with a clinical laboratory and a properly trained technician should be able to diagnose acute amoebic dysentery correctly by detecting motile trophozoites with ingested red blood cells and by the low number of pus cells (usually less than 10 per high-power field) in freshly voided stools. Such cases can be treated properly with metronidazole or another nitroimidazole compound and their response to treatment observed. Cases of liver abscess can also be better diagnosed and managed in hospitals.

Asymptomatic infections are occasionally diagnosed in a hospital, health centre or epidemiological laboratory with facilities for microscopic examinations. In an endemic area, the consensus is not to treat such persons because the probability of reinfection is very high. There may, however, be an epidemiological reason for treating them, e.g., high prevalence of potentially pathogenic strains in the area, or the carrier is a food handler by profession. In a non-endemic area, these patients are always likely to be treated.

Epidemic control

Amoebiasis characteristically occurs in endemic forms in areas of high prevalence, probably as a result of high levels of transmission and constant reinfection in crowded populations living in insanitary conditions. Epidemic outbreaks are rare and are usually associated with sewage seepage into the water supply. Prompt treatment of symptomatic cases and environmental hygienic interventions should help in combating outbreaks.

Chemoprophylaxis and immunization

At present there is no acceptable chemoprophylaxis for amoebiasis, even for non-immune travellers. Mass examination followed by selective chemotherapy—i.e., treatment of all infected persons—cannot be considered a solution for the control of amoebiasis.

The induction of protective immunity is at an experimental stage. It has been shown that rodents can be immunized against intrahepatic challenge with pathogenic strains, using live trophozoites as well as crude or purified *E. histolytica* antigens. Furthermore, cellular immune responses have been induced in primates by the administration of purified *E. histolytica* antigen. No undesirable reactions have been registered in the different animal species immunized. These facts indicate that it should be possible to develop an antiamoebic vaccine (*3*).

RESEARCH PRIORITIES FOR THE DEVELOPMENT OF IMPROVED CONTROL STRATEGIES

Simplified reliable diagnostic and survey techniques

There is a need to develop, for general use, simple and reliable techniques for the identification of *E. histolytica*, and for the preservation of faecal samples and their transportation to a reference laboratory for further examination.

In view of the recent developments in the immunology of amoebiasis, an evaluation should be made of the various immunodiagnostic techniques now available for the detection of serum antigen, faecal antigen, serum antibodies (especially IgM antibodies), and immune complexes. The development of a standardized diagnostic test for general use should be promoted and supported. Serious consideration should in particular be given to promoting a collaborative study, using standardized methods and reagents, to evaluate the radioimmunoassay and ELISA techniques as methods of detecting amoebic protein antigen in serum and to determine their usefulness for the diagnosis of invasive amoebiasis.

The production and evaluation of monoclonal antibodies for the detection of *E. histolytica* antigen in serum and faeces should be supported.

Community surveys

Community surveys, utilizing appropriate techniques, should be carried out in endemic foci to determine the clinical and epidemiological features of amoebiasis. In particular, studies should be undertaken to confirm or refute the impression that illness is more severe in certain age groups, geographical regions, and ethnic groups.

Longitudinal studies to assess the course of intestinal amoebiasis, the proportion of symptomatic and asymptomatic infections in defined communities, the mode of transmission and seasonality, and also possible host factors (e.g., immune status, undernutrition, etc.) deserve consideration.

Studies on pathogenesis

Using the electrophoretic isoenzyme technique, *E. histolytica* isolates from asymptomatic and symptomatic individuals in various geographical areas should continue to be studied to confirm the relationship between the zymodeme pattern and the clinical expression of illness. These studies may be combined with community surveys.

Further studies are required on the pathogenesis of human invasive amoebiasis. Studies of the role of immune complexes in amoebiasis should also be encouraged. Further efforts are needed to develop suitable experimental animal models simulating human disease.

Clinical management

The development of effective but inexpensive new drugs and/or new formulations for both invasive and luminal amoebiasis should be encouraged. A single-dose regimen will be particularly beneficial.

Immunological studies

Research aimed at the antigenic characterization of amoebae of different zymodeme groups should be promoted. Basic studies on the role of humoral and cell-mediated immunity are essential.

Research strengthening

Efforts should be made to identify research workers and institutions in developing countries that could carry out operational and biomedical research on amoebiasis, and to provide adequate support to enable them to work independently or in collaboration with scientists in the developed world.

CONCLUSIONS AND RECOMMENDATIONS

Despite the recognition of the problem caused by the high morbidity and mortality due to amoebiasis, and the serious need for knowledge in this field, there is a paucity of interested institutions and research workers. Support from national, international, and other funding organizations is minimal, and a reappraisal of this situation is sorely needed. In addition, laboratory facilities are often insufficient and the training of laboratory personnel inadequate for the diagnosis of amoebiasis. However, the activities related to parasitic diarrhoeas of the WHO Diarrhoeal Diseases Control and Parasitic Diseases Programmes offer new possibilities for improving both research and services in amoebiasis control.

Appropriate strategies currently applicable for control

Amobiasis can be prevented and controlled both by non-specific and specific measures.

Non-specific measures are concerned with (i) improved water supply, excreta disposal, and food safety; (ii) health education; and (iii) general social and economic development. In areas endemic for invasive amoebiasis, the activities at the national, district, or community level relating to control of intestinal infections should deal with amoebiasis as well. The implementation of individual and community preventive measures (e.g., washing of hands, proper excreta disposal) should be an essential part of these activities. It should be realized, however, that measures such as the improvement of water supplies and

sanitation are cost-intensive and are likely to be a long-term undertaking.

Specific measures that should be undertaken when possible are (i) community surveys to monitor the local epidemiological situation with regard to amoebiasis; (ii) improvement of case management, i.e., rapid diagnosis and adequate treatment of patients with invasive amoebiasis at all levels of the health services, including the community and health centre levels; and (iii) surveillance and control of situations that may encourage the further spread of amoebiasis, e.g., refugee camps, contaminated public water sources.

Intensification of training

Training is an essential component of all the specific interventions and should be provided for both medical and paramedical personnel. The areas in which training should be intensified are: clinical diagnosis and management of amoebiasis, laboratory diagnosis, and survey methodologies.

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