.Two groups of amoebae are of medical importance

a) Amoebae of the alimentary canal: The most important of these is Entamoeba histolytica) which causes intestinal and extraintestinal amoebiasis. Amoebae are also present .in the mouth

b) Potentially pathogenic free-Iiving amoebae: Several species of saprophytic amoebae) are found in soil and water. Two of these, Naegleria and Acanthamoeba are of clinical .interest because they can cause eye infections and fatal meningoencephalitis



Amoebae are structurally simple protozoa which have no fixed shape. They are classified under the Phylum-Sarcomastigophora, Subphylum-Sarcodina, SuperclassRhizopoda, Order-Amoebida. The cytoplasm is bounded by a unit membrane and can be differentiated into an outer ectoplasm and an inner endoplasm. Pseudopodia are formed by the ectoplasm thrusting out, being followed by the endoplasm flowing ,in, to produce blunt projections. Pseudopodial processes appear and disappear producing quick changes in the shape of the cell. These are employed for locomotion .and engulfment of food by phagocytosis. Amoebae may be free-Iiving or parasitic

A few of the free-living amoebae can, on occasion act as human pathogens, producing meningoencephalitis and other infections. Some of them can act as carriers of .pathogenic bacteria. The parasitic amoebae inhabit the alimentary canal

PARASITIC AMOEBAE

:Parasitic amoebae belong to the following genera

Genus Species

١. Entamoeba E.histolytica, E.hartmanni, E.coli, E.polecki

٢. Endolimax E.nana

٣. Iodamoeba I.butschlii

٤. Dientamoeba D.fragilis(now classified as Amoeboflagellate(

Entamoeba histolyticais an important human pathogen, causing amoebic dysentery as well as hepatic amoebiasis and other extraintestinal lesions. E.hartmanniis nonpathogenic, though it resembles E. histolyticavery closely except for its smaller

size and was therefore known as the ‘small race’ of E. histolytica. E.poleckia natural .parasite of pigs and monkeys may sometimes infect humans causing diarrhoea

E. coliis a common commensal in the colon and its importance is that it may be mistaken for E.histolytica. E.gingivalisis present in the mouth, being found in large numbers when the oral hygiene is poor. It has no cystic stage and so the trophozoites depend for transmission on direct oral contact as in kissing, air-borne spread through salivary droplets and fomites such as shared drinking and eating utensils. It is



generally nonpathogenic, though it has been claimed that it contributes to periodontal .disease

All the genera of intestinal amoebae other than Entamoeba are nonpathogenic commensals, except D.fragilis, which may occasionally cause chronic, but mild intestinal symptoms. Intestinal amoebae can be differentiated based on their morphological .features

ENTAMOEBA HISTOLYTICA

Geographical Distribution

E. histolyticais world-wide in prevalence. It is much more common in the tropics than elsewhere, but it has been found wherever sanitation is poor, in all climatic

Morphology

.(E. histolytica occurs in three forms—the trophozoite, precystic and cystic stages (Fig.3.1

Trophozoite

The trophozoite or the vegetative form is the growing or feeding stage of the parasite. It is irregular in shape and varies in size from about 12 to 60 µm. It is large and actively motile in freshly passed dysenteric stools, while in convalescents



FIGURE 3.1: Entamoeba histolytica.(A) Trophozoite; (B) Precystic stage; (C) Uninucleate cyst; ((D

Binucleate cyst; (E) Mature quadrinucleate cyst; 1—Ectoplasm; 2—Endoplasm; 3—Ingested erythrocytes; 4—Pseudopodium; 5—Nucleus; 6—Chromidial bar; 7—Glycogen mass

and carriers, it is much smaller. The parasite as it occurs free in the lumen as a

commensal is generally smaller in size, about 15 to 20 µm and has been called the .minutaform

,The protoplasm is differentiated into a thin outer layer of clear, transparent refractive ectoplasm and an inner finely granular endoplasm having a ground glass appearance. Pseudopodia are formed by a sudden thrusting movement of the .ectoplasm in one direction, followed by the streaming in of the whole endoplasm The direction of movement may be changed suddenly, with another pseudopodium being formed at a different site, when the whole cytoplasm flows in the direction of the new pseudopodium. Typical amoeboid motility is a crawling or gliding movement and not a free-swimming one. The cell has to be attached to some surface or particle for it to move. In culture tubes, the trophozoites may be seen crawling up the side of the glass tube. Pseudopodium formation and motility are inhibited .at low temperatures

The endoplasm contains the nucleus, food vacuoles and granules. The nucleus is not clearly seen in the living trophozoite, but can be distinctly demonstrated in preparations stained with iron-haematoxylin or Gomorri's trichrome stains. The nucleus is spherical, 4 to 6 µm in size and contains a small central karyosome surrounded by a clear halo. The karyosome is anchored to the inner surface of the nuclear membrane '.by fine radiating fibrils called the linin network giving a 'cartwheel appearance

The delicate nuclear membrane is lined by a rim of chromatin distributed evenly .as small granules

The trophozoites from acute dysenteric stools often contain phagocytosed erythrocytes. This feature is diagnostic as phagocytosed red cells are not found in any other .commensal intestinal amoebae

The trophozoite divides by binary fission once in about 8 hours. Trophozoites ,are delicate organisms and are killed by drying, heat and chemical disinfectants ,They do not survive for any length of time in stools outside the body. Therefore the infection is not transmitted by trophozoites. Even if live trophozoites from freshly passed stools are ingested, they are rapidly destroyed in the stomach and cannot .initiate infection

Precystic Stage

Some trophozoites undergo encystment in the intestinal lumen. Encystment does not occur in the tissues nor in feces outside the body. Before encystment the trophozoite extrudes its food vacuoles and becomes round or ovoid about 10 to ٢٠ µm in size. This is the precystic stage of the parasite. It secretes a highly refractile .cyst wall around it and becomes the cyst

Cystic Stage

The cyst is spherical, about 10 to 20 µm in size. The early cyst contains a single nucleus and two other structures—a mass of glycogen and one to four chromatoid bodies or chromidial bars, which are cigar-shaped or oblong refractile rods with rounded ends. The chromatoid bodies are so called because they stain with haematoxylin .like chromatin. As the cyst matures, the glycogen mass and chromidial bars disappear The nucleus undergoes two successive mitotic divisions to form two and then four nuclei. The mature cyst is quadrinucleate. The nuclei and chromidial bodies can be made out in unstained films, but they appear more prominently in stained preparations. With iron-haematoxylin stain the nuclear chromatin and the chromatoid bodies appear deep blue-black, while the glycogen mass appears unstained. When stained with iodine the glycogen mass appears golden brown, the nuclear chromatin and karyosome bright yellow and the chromidial bars appear as clear spaces, being .unstained

Life Cycle

The infective form of the parasite is the mature cyst passed in the feces of convalescents .and carriers. The cysts can remain viable under moist conditions for about ten days The cysts ingested in contaminated food or water pass through the stomach undamaged and enter the small intestine. When the surrounding medium becomes alkaline. The cyst wall is damaged by trypsin in the intestine, leading to excystation. The cytoplasm gets detached from the cyst wall and amoeboid movements appear causing a tear in the cyst wall through which the quadrinucleate amoeba emerges. This stage is called the metacyst. The nuclei in the metacyst immediately undergo division to form

eight nuclei, each of which gets surrounded by its own cytoplasm to become eight small amoebulae or metacystic trophozoites. If excystation takes place in the small intestine, the metacystic trophozoites do not colonise there, but are carried to the .caecum

The optimum habitat for the metacystic trophozoites is the caecal mucosa where they lodge in the glandular crypts and undergo binary fission. Some develop into precystic forms and cysts, which are passed in feces to repeat the cycle (Figs 3.2 .(and 3.3

.The entire life cycle is thus completed in one host

Infection with E. histolyticadoes not necessarily lead to disease. Infact, in most cases it remains within the lumen of the large intestine, feeding on the colonic contents



,FIGURE 3.2: Life cycle of E. histolytica. (1) Trophozoite in gut lumen, (2) Precystic form ,Uninucleate cyst, (4) Binucleate cyst, (5) Quadrinucleate cyst, passed in faeces (٣) Mature cyst—infective when ingested, (7) Excystation in small intestine, (8) Metacystic (٦) (form, (9) Eight daughter amoebulae, (10) Trophozoite shed in faeces—cannot encyst, (11 Tissue form of trophozoite in colonic ulcer—shows ingested erythrocytes

(FIGURE 3.3: Life cycle of E. histolytica(Schematic



and mucus as a commensal without causing any ill effects. Such persons become carriers or asymptomatic cyst passers, as their stool contains cysts. They are responsible for the maintenance and spread of infection in the community. The infection may get spontaneously eliminated in many of them. Sometimes, the infection may be activated and clinical disease ensues. Such latency and reactivation are characteristic .of amoebiasis

Culture

Boeck and Drbohlav reported the successful cultivation of E.histolyticain 1925 using an egg slant-Locke's solution diphasic medium. A monophasic liquid medium was described by Balamuth in 1946. Robinson's medium has been widely used for cultivation of amoebae. In these media and their modifications, amoebae grow only

in presence of enteric bacteria or other protozoa and starch or other particles. Axenic ,cultivation which does not require the presence of other microorganisms or particles was first developed by Diamond in 1961. This yields pure growth of the amoeba and has been very useful for physiological, immunological and pathogenicity studies .of amoebae

Pathogenicity

The lumen dwelling amoebae do not cause any illness. Only when they invade the intestinal tissues do they cause disease. This happens only in about 10 per cent of cases of infection, the remaining 90 per cent being asymptomatic. The factors that .determine tissue invasion are not fully understood

Not all strains of E.histolyticaare pathogenic or invasive. All strains can adhere to host cells and induce proteolysis of host cell contents in vitro but only pathogenic strains can do so in vivo. Differentiation between pathogenic (P) and nonpathogenic NP) strains can be made by several methods including susceptibility to complement) mediated lysis and phagocytic activity or by the use of genetic markers or monoclonal antibodies. Amoebic cysteine proteinase which inactivates the complement factor C3 is an important virulence factor of P strains. Based on electrophoretic mobility ,of 6 isoenzymes (acetylglucosaminidase, aldolase, hexokinase, NAD-diaphorase peptidase, phosphoglucomutase), E.histolyticastrains can be classified into at least (zymodemes. Of these only 9 are invasive (P) and the rest are noninvasive (NP ٢٢ commensals. The zymodemes show a geographical distribution. Even in endemic areas, NP zyodemes are far more common than P ones, which account only about .per cent of the total population ١٠

It has been proposed that P and NP strains though morphologically identical may represent two distinct species—the P strains being E.histolytica, and the NP strains .reclassified as E.dispar. Trophozoites of E.disparcontain bacteria, but no RBC

Host factors such as stress, malnutrition, alcoholism, corticosteroid therapy and immunodeficiency may influence the outcome of infection. Some glycoproteins in colonic mucus bind avidly to surface receptors of the amoeba trophozoites, blocking their attachment to epithelial cells. Alteration in the nature and quantity of colonic

mucus may, therefore, influence virulence. Virulence may also be conditioned by .the bacterial flora in the colon

The metacystic trophozoites penetrate the columnar epithelial cells in the crypts of Lieberkuhn in the colon. Penetration is facilitated by the tissue lytic substances released by the amoebae which damage the mucosal epithelium and by the motility of the trophozoite. Mucosal penetration by the amoeba produces discrete ulcers with pinhead centre and raised edges. Sometimes the invasion remains superficial and .confined to the mucosal epithelium leading to erosion which may spread laterally These heal spontaneously without any ill effects. More often, the amoebae make ,their way to the submucosal layer where they multiply rapidly and form colonies destroying the tissues around by lytic necrosis and forming an abscess. The abscess breaks down to form an ulcer. Amoebic ulcer is the typical lesion seen in intestinal amoebiasis. The ulcers are multiple and confined to the colon, being most numerous in the caecum and next in the sigmoido-rectal region. The intervening mucous .membrane between the ulcers remains healthy

Ulcers appear initially on the mucosa as raised nodules with pouting edges. They later break down discharging brownish necrotic material containing large numbers of trophozoites. The typical amoebic ulcer is flask-shaped in cross section, with mouth and neck being narrow and the base large and rounded. Multiple ulcers may coalesce to form large necrotic lesions with ragged or undermined edges and covered with brownish slough. The ulcers generally do not extend deeper than the submucous layer, but amoebae spread laterally in the submucosa causing extensive undermining and patchy mucosal loss. Amoebae are seen at the periphery of the lesions and extending into the surrounding healthy tissues. Occasionally, the ulcers may involve the muscular and serous coats of the colon, causing perforation and peritonitis. Blood .vessel erosion may cause haemorrhage

The superficial lesions generally heal without scarring, but the deep ulcers form scars .which may lead to strictures, partial obstruction and thickening of the gut wall

Occasionally, a granulomatous growth may develop on the intestinal wall from a chronic

.ulcer. This amoebic granuloma or amoebomamay be mistaken for a malignant tumour

During its invasion of the intestinal wall, amoebae often penetrate radicles of the portal vein and are transported through the portal circulation to the liver. Most ,of them fail to lodge, but some manage to get established in the hepatic lobules .where they multiply and initiate lytic necrosis with little inflammatory reaction

Hepatic invasion is multifocal, the right lobe being more affected. With increasing size of the lesions and continuing necrosis, there occurs considerable leucocytic infiltration. .There is also an enlargement of the liver. This stage is known as amoebic hepatitis

One or more of the lesions in the liver may extend peripherally to develop into .amoebic abscesses. Which may vary in size from a few millimeters to several centimeters ('The centre of the abscess contains thick chocolate brown pus ('anchovy sauce pus .which is liquefied necrotic liver tissue. It is bacteriologically sterile and free of amoebae

Immediately surrounding the central necrotic area is a median zone consisting only of coarse stroma. At the periphery is almost normal liver tissue, which contains invading amoebae. If the abscess has developed rapidly, there may be no limiting



capsule other than liver tissue, but more chronic lesions are surrounded by a fibrous wall. Liver abscess may be multiple or more often solitary,usually located in the upper right lobe of the liver. Jaundice develops only when lesions are multiple or when they press on the biliary tract. Untreated abscesses tend to rupture into the ,adjacent tissues and organs, through the diaphragm into the lung or pleural cavity into the pericardium, peritoneal cavity, stomach, intestine or inferior vena cava, or .externally through the abdominal wall and skin

Very rarely, amoebiasis of the lung may occur by direct haematogenous spread from the colon, without hepatic involvement, but it is most often due to direct extension from the liver by an abscess rupturing through the diaphragm. It is, therefore, most ,common in the lower part of the right lung. A hepatobronchial fistula usually results with expectoration of chocolate brown sputum. Less often, an amoebic empyema .develops

FIGURE 3.4: Sites affected in amoebiasis

Involvement of distant organs is by haematogenous spread. Instances are abscesses .in the brain, spleen, adrenals and kidneys

Cutaneous amoebiasis is by direct spread, from the rectum perianally and from colostomy openings and sinuses draining liver abscesses. Extensive necrosis and sloughing occur. Trophozoites can be demonstrated in the lesions. It can also occur .(as a venereal infection of the penis following anal intercourse (Fig. 3.4

Clinical Features

The incubation period is highly variable, from 4 days to a year or longer. On an average it is from 1 to 4 months. The clinical course is characterised by prolonged .latency, relapses and intermissions



Amoebiasis can present in different forms and degrees of severity depending on the organ affected and the extent of damage caused. It can be classified as intestinal .and extraintestinal amoebiasis

Intestinal Amoebiasis

The clinical picture covers a wide spectrum from noninvasive carrier state to fulminant .colitis

The typical manifestation of intestinal amoebiasis is amoebic dysentery. This may resemble bacillary dysentery, but can be differentiated on clinical and laboratory grounds. Compared to bacillary dysentery, it is usually insidious in onset and the .abdominal tenderness less and localised

The stools are large, foul smelling and brownish black, often with bloodstreaked mucus intermingled with faeces. The red blood cells in stools are clumped and reddish .brown in colour. Cellular exudate is scanty . Charcot-Leyden crystals are often present E.histolyticatrophozoites can be seen containing ingested erythrocytes. The patient is usually afebrile and nontoxic. In fulminant colitis there is confluent ulceration and .necrosis of colon. The patient is febrile and toxic

Intestinal amoebiasis does not always result in dysentery. Quite often there may be only diarrhoea or vague abdominal symptoms popularly called ‘uncomfortable belly’ or ‘growling abdomen.’ Chronic involvement of the caecum causes a condition

.simulating appendicitis

Extraintestinal Amoebiasis

.Hepatic involvement is the most common extraintestinal complication of amoebiasis

Though trophozoites reach the liver in most cases of amoebic dysenterys, only in a small proportion do they manage to lodge and multiply there. Several patients with amoebic colitis develop an enlarged tender liver without detectable impairment of liver function or fever. This acute hepatic involvement (amoebic hepatitis) may be due to repeated invasion by amoebae from an active colonic infection or to toxic substances from the colon reaching the liver. It is probable that liver damage may be caused not directly by the amoebae, but by lysosomal enzymes and cytokines from the inflammatory cells surrounding the trophozoites. In about 5 to 10 per cent of persons with intestinal amoebiasis, liver abscess may ensue. It is more common in adult males. The patient feels heaviness and pain in the liver area and referred .pain around the right shoulder. Fever with chills is common, as also weight loss

.Jaundice is not common

Pleuropulmonary amoebiasis usually follows extension of hepatic abscess through .the diaphragm and therefore, the lower part of the right lung is the usual area affected

Very rarely, abscess formation may occur at any site on either lung due to haematogenous spread. The abscess draining into a bronchus leads to reddish brown pus

.being coughed out

Amoebic abscess of the brain may occasionally result from haematogenous spread from amoebic lesions in the colon or other sites. It causes severe destruction of brain



tissue and is fatal. Abscesses in other organs such as spleen, kidney and suprarenal .gland are rare and follow blood spread

Cutaneous amoebiasis occurs by direct extension around the anus, colostomy site or discharging sinuses from amoebic abscesses. Extensive gangrenous destruction .of the skin occurs. The lesion may be mistaken for condylomata or epithelioma

The prepuce and glans are affected in penile amoebiasis which is acquired through anal intercourse. Similar lesions in females may occur on vulva, vaginal wall or cervix

.by spread from perineum. The destructive ulcerative lesions resemble carcinoma

Laboratory Diagnosis

Definitive diagnosis of amoebiasis depends on the demonstration of E.histolytica trophozoites or its cysts in stools, tissues or discharges from the lesions. Cultures are not employed for routine diagnosis. Immunological tests are not helpful for .diagnosis of intestinal infection but may be of use in extraintestinal amoebiasis

Intestinal Amoebiasis

Acute amoebic dysentery: The disease has to be differentiated from bacillary dysentery Table 3.1). The stool sample has to be collected directly into a wide mouthed container) ,and examined without delay. Prior administration of antiamoebic drugs, bismuth kaolin or mineral oil may interfere with demonstration of the trophozoite. It should be inspected for macroscopic and microscopic features, as well as routine examination .for other parasites also. Examination of three separate samples is recommended

a. Macroscopic appearance: The stool is copious, semiliquid, brownish black in colour .and contains foul smelling faecal material intermingled with blood and mucus .It is acid in reaction. It does not adhere to the container

b.Microscopic appearance: The cellular exudate is scanty and consists of a few pus cells, epithelial cells and macrophages. The red cells are aggregated and yellowish or brownish red in colour. Charcot-Leyden crystals are often present. But this finding is only suggestive, because they may also occur in some other bowe1 disorders such as ulcerative colitis and malignancy. In freshly passed motion unmixed with urine or antiseptics, actively motile trophozoites of E.histolytica can be demonstrated in unstained saline mounts.The presence of ingested erythrocytes clinches the identity of E.histolytica, as they are not found in any other intestinal amoeba. Stained films may not be necessary as a routine for diagnosis in acute cases, but trichrome or iron-haematoxylin stained films provide .(the most dependable identification and differentiation (Fig. 3.5

Culture and serology are not routinely employed. Serology is usually negative .in early cases and in the absence of deep invasion

Chronic Amoebiasis and Carriers

Sigmoidoscopy may show amoebic ulcers in the colon, from which biopsy tissue may be taken for direct microscopy and histopathology. Identification of asymptomatic



FIGURE 3.5

carriers is important in epidemiological survey and in screening persons employed .in food handling occupations

,In chronic patients, convalescents and carriers, besides naturally passed stools it may be necessary to examine stools obtained after a saline purge for trophozoites and cysts. Excretion of amoeba is irregular and repeated stool examination is therefore -necessary. The demonstration of cysts is facilitated by the use of a suitable concen



FIGURE 3.6: Note the characteristic nuclear structure of the different amoebae (parasitic to man. (Heidenhain's haematoxylin Magn. x 2000



Table 3.1: Differential features of amoebic and bacillary dysentery

| Features | Amoebic dysentery | Bacillary dysentery |
|---|---|---|
| Clinical | | |
| Onset | Slow | Acute |
| Fever | Absent | Present |
| Toxicity | Absent | Present |
| Abdominal tenderness | Localised | Generalised |
| Tenesmus | Absent | Present |
| Stool | | |
| Frequency | 6-8 per day | Over 10 per day |
| Odour | Offensive | Nil |
| Colour | Dark red | Bright red |
| Nature | Faeces mixed with blood and mucus with blood and mucus | little or no faeces |
| Consistency | Not adherent | Adherent to container |
| Reaction | Acid | Alkaline |

Microscopy

Cellular exudate Scanty Abundant

,Red blood cells Clumped Discrete or in rouleaux

yellowish brown bright red

Macrophage Few Several, some with

ingested red blood cells

Eosinophils Present Absent

Charcot-Leyden crystals Present Absent

Motile bacteria Present Absent

Amoeba Motile trophozoites Absent

with ingested red

blood cells

tration method such as the zinc sulphate centrifugal floatation technique. Examination of iodine and iron-haematoxylin stained preparations is helpful. Trophozoites, when .present may be in the minuta form and may not have ingested erythrocytes Differentiation from other amoebae may require the study of nuclear morphology after staining. Samples may be fixed with 10 per cent formalin, Schaudin's fixative or polyvinyl alcohol and stained with Gomorri trichrome or periodic acid Schiff .stains. The differential characters of intestinal entamoebae are shown in Table 3.2 Cultures are not used routinely but may on occasion prove positive in cases found negative by microscopy. Cultures permit the determination of zymodeme patterns for differentiation between pathogenic and nonpathogenic strains. Serological tests .may not be positive except in cases of invasive amoebiasis

Immunodetection tests for identifying E.histolyticaantigens in clinical samples are available. Highly specific ELISA reagents can differentiate between E.histolyticaand ,E.disparantigens. Polyvalent immunochromatographic strip tests can detect amoeba .giardia and cryptosporidium antigens in stool samples

Extraintestinal (Invasive) Amoebiasis

Hepatic amoebiasis:In diffuse hepatic amoebiasis (amoebic hepatitis) without localised abscess formation, laboratory diagnosis may be difficult. Often stool examination

Table 3.2: Differential features of intestinal entamoebae

E.histolytica E.hartmanni E.coli

Trophozoite

Size (µm) 12-60 4-12 20-50

Motility Active Active Sluggish

Pseudopodia Finger shaped, Finger shaped Short, blunt

rapidly extruded rapidly extruded slowly extruded

Cytoplasm Clearly defined into Clearly defined into Differentiation

ectoplasm and endoplasm ectoplasm and endoplasm not distinct

Inclusions Red blood cells present Bacteria and other Bacteria and other

no bacteria. particles, no RBC particles, no RBC

Nucleus Not clearly visible in Not visible in unstained Visible in unstained

unstained films films films

Karyosome Small, central Small, eccentric Large, eccentric

Nuclear Delicate, with fine Coarse chromatin granules Thick, with coarse

membrane chromatin dots chromatin granules

Cyst

Size (µm) 10-15 5-10 10-30

Nuclei in 4 4 8

mature cyst

Glycogen Seen in uninucleate, but not in quadrinucleate stage Seen up to

mass quadrinucleate stage

Chromidial bars 1-4, with rounded Many, shape Splinter-like with

ends irregular angular ends

is negative for amoebae and a history of dysentery may be absent. In such cases .serological tests can be helpful

.Craig (1928) was the first to report a complement fixation test in amoebiasis Subsequently a number of different serological tests have been developed including indirect haemagglutination (IHA), latex agglutination (LA), gel diffusion precipitation

GDP), cellulose acetate membrane precipitation (CAP) test, counter current ) immunoelectrophoresis (CIE) and enzyme linked immunosorbent assay (ELISA). While lHA

and LA are highly sensitive, they often give false-positive results. They remain positive

for several years even after successful treatment. Gel precipitation tests are less sensitive, but more specific. ELISAs are both sensitive and specific and like GDP and CIE

become negative within six months of successful treatment. Highly sensitive radioimmunoassay (RIA) and DNA probes have been introduced for detection of amoeba

.antigens in blood pus and faeces but these are too complex for routine use

In case of liver abscess when diagnostic aspiration is done the pus obtained from

the centre of the abscess may not contain amoebae as they are confined to the periphery. .The fluid draining after a day or two is more likely to contain the trophozoite

Aspirates from the margins of the abscess also would show the trophozoites. Cysts

.are never seen in extraintestinal lesions

Other Extraintestinal Amoebiasis

In pulmonary amoebiasis the trophozoite may be seen in the expectorated anchovy

sauce sputum. Cutaneous amoebiasis, and whenever accessible materials from other

.invasive lesions also show the trophozoites



Immunity

.Infection with invasive strains induces both humoral and cellular immune responses

Local and systemic antibodies can be demonstrated within a week of invasive

.infection

Infection confers some degree of protection as evidenced by the very low frequency

of recurrence of invasive colitis and liver abscess in endemic areas. The course and

severity of amoebiasis do not seem to be affected by HIV infection. Serological

.response is hardly ever seen in infection with noninvasive zymodemes

Epidemiology

Amoebiasis is worldwide in prevalence though it is more common in the tropical

areas where sanitation is poor. Prevalence rates vary from as low as 1 per cent in

affluent countries to more than 50 per cent in some developing countries. Some 500

million new infections occur each year worldwide. Infection occurs at all ages and

in both sexes, though it is more common in adults than in children, and in males .than in females

The source of infection is a carrier or asymptomatic cyst passer. The patient with acute dysentery is of no importance in transmission as the stools then contain only .trophozoites which are not infective. Carriers may shed the infective cysts for years When cooks and other food handlers happen to be carriers they can transmit the infection readily. Amoebiasis in animals does not appear to be of any importance .as a source of human infection

Amoebiasis is essentially an endemic disease though it can occasionally occur in epidemic form due to contamination of water sources. Contaminated food and water .constitute the most important vehicles of infection

The cysts are relatively resistant. They can survive for several months in water at 0°C, 3 days at 30°C, 30 minutes at 40°C and 5 minutes at 50°C. In grossly contaminated water and sewage they may survive longer. They remain viable in moist soil for upto 10 days. They can resist 1/2500 mercury bichloride, 5 per cent HCl or 0.5 .per cent formalin for 30 minutes and 1/500 potassium permanganate for 1 to 2 days

They are killed by boiling, desiccation, freezing to below—5°C, 1/20 cresol in 30 minutes and 5 per cent acetic acid in 15 minutes. Ordinary residual chlorination of .water may not destroy them, though super-chlorination does

Flies and cockroaches may act as mechanical vectors. Viable cysts have been found .in their droppings for a day or two after exposure

Increased and varied male homosexual practices, particularly in the West, have enhanced the incidence of amoebiasis, which has been recognised as a ‘gay bowel ’.disease

Prophylaxis

General prophylaxis is as for all faecal-oral infections. Food and water have to be protected from contamination with human excreta. Detection and treatment of carriers .and their exclusion from food handling occupations limit the spread of infection .Health education and inculcation of healthy personal habits help in control

Treatment

Two classes of drugs are used in the treatment of amoebiasis—the luminal amoebicides e.g. diloxanide furoate, iodoquinol, paromomycin, tetracycline) acting in the intestinal) (lumen, but not in tissues, and the tissue amoebicides (e.g. emetine, chloroquine effective in systemic infection, but less so in the intestine. Metronidazole and related compounds act at both sites. Emetine, for long the sheet anchor in treatment of .amoebiasis has largely been given up because of its toxicity

Opinion is divided about the need for treating asymptomatic cysts passers in .endemic areas. It may perhaps be futile in view of the high rate of reinfection

ENTAMOEBA HARTMANNI

Entamoeba hartmannioccurs wherever E.histolyticais found and was till recently considered to be a "small race" of the latter. It was quite often mistaken for E.histolytica and reported as such. It is now considered to be a separate species of nonpathogenic commensal intestinal amoeba. It is much smaller than E.histolytica, the trophozoite measuring 4 to 12 µm and cyst 5 to 10 µm in size. Trophozoites do not ingest red .cells and their motility is less vigorous. The cyst resembles that of E.nana

ENTAMOEBA COLI

Entamoeba coliwas first described by Lewis (1870) and Cunningham (1871) in Calcutta and its presence in healthy persons was reported by Grassi (1878). It is worldwide in distribution. It is a nonpathogenic commensal intestinal amoeba. Its medical importance is that it has to be differentiated from E.histolytica. It is larger, about .to 50 µm with sluggish motility and contains ingested bacteria but not red cells ٢٠

The nucleus is clearly visible in unstained films and has a large eccentric karyosome ,and thick nuclear membrane lined with coarse granules of chromatin. Cysts are large to 30 µm in size, with a prominent glycogen mass in the early stage. The chromatoid ١٠ bodies are splinter like and irregular. The mature cyst has eight nuclei. The life cycle is the same as in E.histolyticaexcept that it remains a luminal commensal without .tissue invasion and is nonpathogenic

ENTAMOEBA POLECKI

Originally described as an intestinal parasite of pigs and monkeys, E.poleckihas been

detected in the human intestine in some parts of South East Asia, particularly in Papua-New Guinea, where it is a common intestinal commensal. However, it does .not appear to be a significant human pathogen

The trophozoite resembles that of E.coli. The cyst is uninuclear, with many .prominent pointed chromidial bars and one or more large nonglycogen inclusions

ENTAMOEBA GINGIVALIS

Entamoeba gingivaliswas discovered by Gros in 1849 and so was the first amoeba of humans to have been described. It is global in distribution. Only the trophozoite



is found, the cystic stage being apparently absent. The trophozoite is about 10 to µm, actively motile with multiple pseudopodia. The cytoplasm contains food ٢٠ vacuoles with ingested bacteria, leucocytes and epithelial cells. The presence of ingested leucocytes and their nuclear fragments is diagnostic as no other amoeba ingests these cells. The nucleus is round, with a delicate central karyosome and nuclear membrane .lined with coarse chromatin granules

.The amoeba lives in the gingival tissues and is abundant in unhygienic mouths

It is a commensal and is not considered to cause any disease. It is transmitted by .direct oral contact, through droplets of saliva or fomites

E.gingivalishas been found in bronchial washings from cases of pulmonary .suppuration and in sputum, where it can be mistaken for E.histolyticafrom lung abscess

The amoeba has been reported in vaginal and cervical smears of women using intrauterine devices and they disappear spontaneously with the removal of these .devices. Similar amoebae have been observed in the mouth of dogs, cats and monkeys

ENDOLIMAX NANA

.This common commensal amoeba is widely distributed. It lives in the human intestine

The trophozoite is small (nana, meaning small), less than 10 µm in size, with a slow slug like motility.The nucleus has a conspicuous eccentric karyosome connected to the nuclear membrane by one or more coarse stands. The cyst is small, oval and .tetranucleate with the glycogen mass and chromidial bars inconspicuous or absent

.It is nonpathogenic

IODAMOEBA BUTSCHLII

This is widely distributed, though less common than E.coliand E.nana. The trophozoite is small, 6 to 12 µm, with a conspicuous nucleus. The prominent karyosome is half ,the size of the nucleus and surrounded by refractile globules. The cyst is oval .(uninucleate and has a prominent iodine-staining glycogen mass (iodophilic body .Hence the name 'Iodamoeba.' It is nonpathogenic

DIENTAMOEBA FRAGILIS

Dientamoeba fragilis, long considered an amoeba has been reclassified as an aberrant .trichomonad flagellate (amoeboflagellate) based on electron microscopic features

The trophozoite is 7 to 12 µm in diameter. It is motile with broad hyaline leaflike .pseudopodia. They have 1 to 4 nuclei, the binucleate form being the most common

The name Dientamoeba fragilisrefers to the binucleate feature and the fragile nature ,of its cytoplasm. The nuclear chromatin is present as 3 to 5 granules in the centre .with no peripheral chromatin on the nuclear membrane. D. fragilishas no cyst stage

It is seen worldwide and is reported to be the most common intestinal protozoan parasite in Canada. It lives in colonic mucosal crypts, feeding on bacteria. It does ,not invade tissues, but may rarely ingest RBCs. Formerly believed to be nonpathogenic ,it has now been associated with a variety of symptoms like intermittent diarrhoea



abdominal pain, flatulence and fatigue. Metronidazole, iodoquinol, paromomycin and tetracycline have been used for treatment. In the absence of a cyst stage, its mode of transmission is not clear. It has been proposed that trophozoites shed in feces may survive in enterobius, ascaris or other nematode eggs and be transmitted .through them

Definitive diagnosis depends on demonstration of the characteristic nuclear structure in permanently stained films. Examination of unstained fecal smears is not .satisfactory

The comparative morphologies of amoebae infecting man is shown in (Figs 3.6 .(and 3.7

PATHOGENIC FREE-LIVING AMOEBAE

Among the numerous types of free-living amoebae found in water and soil, a few are potentially pathogenic and can cause human infections. The first of these to have been recognised was primary amoebic meningoencephalitis(PAM) caused by the amoeboflagellate Naegleria. Acanthamoebaehave been found to cause two diseases, granulomatous amoebic encephalitis(GAE) and chronic amoebic keratitis(CAK). A few instances of GAE caused by leptomyxidamoebae have also been reported. While PAM and CAK occur in previously healthy individuals, GAE has been associated with immunodeficient .states

The term amphizoichas been used for organisms such as these, which can multiply .(both in the body of a host (endozoic) and in free-living (exozoic) conditions (Fig. 3.8

NAEGLERIA

,Naegleria fowleri, the only pathogenic species of naegleria is named after Fowler who with Carter described it first from Australia in 1965. It is found worldwide in warm .fresh waters

N. fowlerihas 3 stages in its life cycle—a dormant cyst form, an amoeboid .(trophozoite form and a flagellate form (hence classified as an amoeboflagellate ,(The amoeboid form is about 10 to 20 µm, showing rounded pseudopodia (lobopodia ,a spherical nucleus with a big endosome, and pulsating vacuoles. This is the feeding .growing and replicating form, seen on the surface of vegetation, mud and water .In water, some of them get transformed into a 'pear-shaped form' with 2 flagella This rapidly motile flagellate form is the main infective stage, more so than the trophozoite. The flagellate can revert to the amoeboid form. Cysts develop from the trophozoites and are seen in the same locations as trophozoites. The cysts are spherical. They are the resting dormant form and can resist unfavourable conditions such as drying and chlorine up to 50 ppm. The trophozoites can withstand moderate .heat (45°C), but die at chlorine levels of 2 ppm and salinity of 0.7 per cent

Human infection comes from water containing the amoebae and usually follows swimming or diving in ponds. Patients are mostly previously healthy young adults or children. The amoebae invade the nasal mucosa, pass through the olfactory nerve branches in the cribriform plate into the meninges and brain to initiate an acute

,FIGURE 3.7: Comparative morphology of amoebae infecting humans

showing trophozoite and cyst stages, as well as enlarged representation of their nuclear structure

purulent meningitis and encephalitis (primary amoebic meningoencephalitis). The

incubation period is 2 days to 2 weeks. The disease almost always ends fatally within

a week. Over 200 cases of PAM have been reported from many countries, including

.India. Most cases have been from the developed countries



FIGURE 3.8: Pathogenic free-living amoebae. (A) Naegleria fowleri:1—Amoeboid trophozoite

showing lobopodia, nucleus with large endosome, and vacuoles. 2—Cyst. 3—Pear-shaped

flagellate form showing flagella. (B) Acanthamoeba culbertsoni: 1—Trophozoite, showing spinous

acanthopodia. 2—Cyst

Diagnosis can be made by CSF examination. The fluid is cloudy to purulent, with

prominent neutrophil leucocytosis, elevated proteins and low glucose, resembling

pyogenic meningitis. Failure to find bacteria in such specimens should raise the

possibility of PAM. Wet film examination of CSF may show the trophozoites. Cysts

are never seen CSF or brain. At autopsy, trophozoites can be demonstrated in brain

histologically.Culture can be obtained in agar seeded with Escherichia coli or in the

.usual cell cultures used for virus isolation. Both trophozoites and cysts occur in culture

.Amphotericin B has been used in treatment with limited success

ACANTHAMOEBA SP

Acanthamoeba culbertsoni(formerly Hartmanella culbertsoni) is the species most often

responsible for human infection, but other species such as A. polyphaga. A.castellani

.and A.astromyxishave also been reported

The trophozoite is large, 20 to 50 µm in size and characterised by spine-like

pseudopodia (acanthopodia). It differs from Naegleria in not having a flagellate stage

.and in forming cysts in tissues. The polygonal double walled cysts are highly resistant

.The cysts are present in all types of environment all over the world

Infection can be acquired by inhalation, ingestion or through traumatised skin

—or eyes, from contaminated water. It presents chiefly as two chronic conditions ,keratitis and encephalitis. Granulomatous lesions in other sites such as skin, lungs .middle ear have also been reported



Chronic amoebic keratitis or keratouveitis, of which over a thousand cases have been reported, develops from the entry of the amoebic cyst through abrasions on the cornea. The large majority of such cases have been associated with the use of contact lenses. The picture resembles that of severe herpetic keratitis with a slow .relapsing course, but the eye is severely painful in the amoebic infection

,Diagnosis is by demonstration of the cyst in corneal scrapings by wet mount histology and by culture. Growth can be obtained from corneal scarpings inoculated .on nutrient agar, overlaid with live or dead Escherichia coliand incubated at 30°C

Treatment with drugs such as propamidine, polyhexamethylene biguanide, chlorhexidine .and ketoconazole, along with surgical procedures has been found useful

Granulomatous amoebic encephalitis is believed to follow inhalation of the dried cysts. In persons with predisposing factors such as steroid use, alcoholism, diabetes and immunodeficiencies, including AIDS. The incubation period is long and the evolution of the illness slow. The picture is that of an intracraneal space occupying lesion with pareses, seizures and mental deterioration. CSF shows lymphocytic pleocytosis. Acanthamoeba trophozoites and cysts can be demonstrated in brain .biopsy by microscopy, culture and immunofluorescence using monoclonal antibodies

No effective treatment is available. Over a hundred cases have been reported, the .majority of them in the HIV infected

The aetiological agent in a few cases of granulomatous amoebic encephalitis has .been identified as a leptomyxid amoeba Balamuthia mandrillaris

FREE-LIVING AMOEBAE AS ALLERGENS

Naegleria and Acanthamoeba have been claimed to be responsible for allergic pneumonitis. This is believed to be due to inhalation of amoebic antigens derived .from amoebae growing in the humidifiers of air conditioning plants

FREE-LIVING AMOEBAE AS CARRIERS

Some free-living water amoebae may sometimes harbour pathogenic bacteria such as legionella, pseudomonas and cholera vibrios. The bacteria can grow and multiply in the amoebae and survive in the cysts, resisting adverse environments, for example in chlorinated water. This may be significant in hospital infection if the water is contaminated with free-living amoebae. These water amoebae have been shown to be acceptable hosts for Chlamydia pneumoniae, Legionella pneumophila and some .enteroviruses

BLASTOCYSTIS HOMINIS

Blastocystis hominis, a common inhabitant of the human intestine was first identified in 1912. Its taxonomical status and clinical significance still remain unclear. After various suggestions, it is now classified as a protozoon assigned to a new suborder .of Amoebida



—B. hominisis a round cell, 6 to 40 µm in size, characterised by a large membrane .(bound central body, surrounded by a layer of cytoplasm containing nuclei (Fig. 3.9 It can put forth pseudopodia and ingest bacterial and other debris. Reproduction .is by binary fission and sporulation

FIGURE 3.9: Blastocystis hominis

Infection is generally asymptomatic, though unconfirmed claims relate it to various illnesses from diarrhoea to arthritis. Long-term carriers are common. Infection leads .to production of circulating antibodies detectable by ELISA or immunofluorescence The mode of spread is believed to be faecal-oral. Infection is reported to be more .common in those in close contact with animals