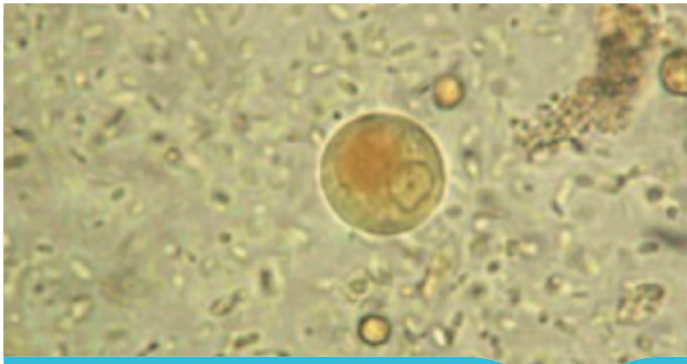




Entamoeba histolytica, *E. histolytica*/*E. dispar*

Family of *Entamoebidae*, group Mycetozoa
Protozoa
Phylum Rhizoflagellata
Parasites

Characteristics and sources of *Entamoeba histolytica*



© Cyst of *E. histolytica*/*E. dispar*

Main microbial characteristics

Entamoeba histolytica, the causative agent of amebiasis (or amoebiasis) is a unicellular eukaryotic pathogen devoid of mitochondria usually ranked among the protists. However, in the phylogenetic classification of living organisms, the family *Entamoebidae*, which includes *E. histolytica* and related species, is currently often assigned to the group of Mycetozoa.

E. histolytica is an exclusively human colon parasite whose cycle comprises two stages:

- trophozoites move in one direction only, replicate in the intestinal lumen and may penetrate the intestinal wall, causing amoebic disease. Abundant in diarrheal stools, trophozoites are poorly resistant in the external environment;
- cysts are eliminated with the faeces of infected individuals and are resistant in the external environment. They then contaminate the environment and are directly infective. They are more common in non-diarrheal stools of patients with chronic amebiasis and carriers with few or no symptoms.

The *Entamoeba histolytica* entity comprises two species: *E. histolytica*, which can invade tissues and is responsible for amebiasis, and *E. dispar*, which is unable to invade tissues and therefore does not cause symptoms.

Since *E. histolytica* and *E. dispar* cysts cannot be distinguished under the microscope, they are known as *E. histolytica*/*E. dispar*. Distinguishing

between the two species requires the detection of specific antigens, or the amplification of specific genomic sequences by PCR. Trophozoites can be identified as *E. histolytica* if they contain phagocytised erythrocytes or if they are observed in biopsies or surgical specimens. Otherwise, they must be considered as *E. histolytica*/*E. dispar*.

Sources of the hazard

Humans infected with *E. histolytica* (patients and healthy carriers) are the only reservoir of the species and the only source of environmental contamination via faecal shedding of cysts. Infection of other mammals (apes, cats, dogs, pigs) is infrequent, as these hosts act as neither reservoir nor source of infection. The lifetime of cysts in the environment varies depending on environmental conditions and can be as much as several months (Table 1). Consequently, the cysts of *E. histolytica* and *E. dispar* can contaminate the environment and persist for several weeks in wastewater and on agricultural products irrigated by it. Water and plants in contact with soil or irrigated by sprinklers are the main environmental sources of the hazard.

Table 1. Cysts of *E. histolytica*/*dispar*: resistance in the environment

Temperature	Environment	Survival
-10°C	Varied	24 hours
0°C – 4°C	Water and sewage sludge	2 to 12 months
10°C – 20°C	Faeces, damp soil, water	10 to 30 days
20°C – 30°C	Water (surface or waste)	< 15 days
28°C – 34°C	Faeces, damp soil, water	8 days
50°C	Varied	Destruction

Transmission routes

The infection is usually contracted by ingesting water or raw fruit or vegetables contaminated by faeces of infected individuals. Direct human-to-human transmission, by contact, may occur frequently particularly since cysts can survive for about 10 minutes on dry skin and 45 minutes under the nails. Amebiasis can also be transmitted through oral-anal sexual practices.

Recommendations for primary production

- Observe the regulations and recommendations in force concerning treatment and spreading of sewage sludge from wastewater, and the use of treated wastewater for irrigation and watering.
- Observe good agricultural practices to minimise the risk of contamination of raw materials (fruit and vegetables) by water from sprinkler irrigation.
- Wastewater treatment and the ban on human fertilizer.

Human foodborne illness

Nature of the disease (Table 2)

Susceptible population groups⁽¹⁾: immunosuppression has rarely been associated with amebiasis. Recent data indicate, first, that seropositive HIV patients (with or without AIDS) have no particular susceptibility to *E. histolytica* and, second, that the course of amoebic infection has no unusual characteristics in these patients.

The occurrence of fulminant necrotising amoebic colitis (very rare) may however be enhanced by corticosteroids, pregnancy, alcoholism and diabetes. Nevertheless, only age (> 60 years) and insufficient levels of potassium in the blood have been identified as independent risk factors.

Dose-effect⁽²⁾ relationships

Infection is possible after ingestion of a single cyst.

Epidemiology

Surveillance system

Little is known of the epidemiology of amebiasis from *E. histolytica* strictly speaking, because most studies do not distinguish between *E. histolytica* and *E. dispar*. Most information thus relates to the *E. histolytica*/*E. dispar* complex. In general it is estimated that approximately 90% of identified strains correspond to *E. dispar*. There is no surveillance system in France, Europe or the United States.

Prevalence

Amebiasis is a parasitic disease linked to poor sanitary conditions, with a global distribution (the climatic factor is not determinative). Endemic areas are extensive (Latin America, Indian subcontinent, Southeast Asia, etc.).

Globally, it is estimated that: (i) more than 500 million people are infected with *E. histolytica*/*E. dispar*, (ii) 100 million people per year have symptomatic amebiasis, (iii) the number of deaths attributable to amebiasis is 100,000 per year. Amebiasis is the third parasitic cause of mortality (WHO).

In Western Europe, a gastroenteritis monitoring plan conducted in the Netherlands (1996 - 1999) showed that *E. histolytica*/*E. dispar* was isolated in 1.1% of symptomatic patients and 0.7% of asymptomatic controls.

In France, there are no general data for the population, but only for occupations at risk, or in patients treated in parasitology. Thus, systematic tests conducted in a population of sewer workers demonstrated carriage in 11% of individuals, whereas these frequencies are much lower in the general population (about 2% of stools examined in hospital outpatients).

Outbreaks

Worldwide, reported outbreaks are mainly waterborne.

Role of food

Main foods to consider

Water is the main vector of foodborne disease. The other foods that are most at risk are raw fruit and vegetables contaminated by irrigation or runoff water polluted by faeces of infected individuals. Epidemic transmission can result from contamination of drinking water, fruit juices, drinks cooled with ice made with contaminated water, ice creams and raw fruit.

Retention and inactivation treatments in industrial environments

Retention treatments

The retention systems used in water treatment are:

- physico-chemical treatments: coagulation-flocculation-separation (sedimentation or flotation) and sand filtration;
- membrane treatments: microfiltration or ultrafiltration.

Given the size of the trophozoites and multinucleated cysts, retention systems can obtain 3 to 4 log₁₀ reductions in the initial load. The turbidity of filtered water should be at most 0.5 or even 0.3 NFU.

(1) Susceptible population group: people with a higher than average probability of developing symptoms of the disease, or severe forms of the disease, after exposure to a foodborne hazard [definition used for the ANSES data sheets].

(2) The relationship between the dose (the amount of microbial cells ingested during a meal) and the effect on an individual.

Table 2. Disease characteristics

Incubation period	Target population	Main clinical forms/symptoms	Duration of symptoms	Duration of shedding of cysts	Complications (including mortality)
Not applicable	Anyone exposed, through ingestion, to viable cysts of <i>E. histolytica</i>	Asymptomatic (90%): no symptoms or only minor digestive disorders in the year following infection	Not applicable	Several months	Colonic and/or visceral amebiasis (4-10%)
2 to 4 weeks		Acute colonic amebiasis (amoebic dysentery): abdominal pain, diarrhoea (often not febrile), with emission of pus, mucus and blood	<10 days with treatment. (Possible evolution to chronic disease)	Several months (up to 4.5x10 ⁷ cysts per day)	Haemorrhage; perforation, peritonitis; ulcerations, necrosis, megacolon
2 to 4 weeks		Chronic colonic amebiasis: recurrent episodes of diarrhoea alternating with moderate gastrointestinal disorders	A few weeks to several months	Several months	Same as above, amoeboma (pseudotumour colitis, reaction to chronic amoebic ulcerations)
2 to 4 weeks		Fulminant necrotising amoebic colitis: profuse bloody diarrhoea, leukocytosis, fever, peritonitis	A few days	Not applicable	Perforation, peritonitis (75%), mortality >40%
2 to 4 weeks, sometimes months or years		Visceral amebiasis (1% of amebiasis cases): extraintestinal amoebic abscesses in the liver (90%)	A few weeks to several months	Not applicable	Pleuropulmonary, pericardial, cerebral, genito-urinary extension, bacterial superinfection. Fatal if untreated. With proper care and without complications, mortality is 1 to 3%.

Inactivation treatments

Disinfectants

Data on the action of disinfectants are limited. Chemical treatments at the CT* values normally applied in the treatment of water intended for human consumption are not effective against cysts. Higher concentrations or longer contact times are required, i.e. a CT of 15 to 30 for hypochlorites, 10 to 20 for chlorine dioxide and 2 to 5 for ozone.

Table 3. Activity of disinfectants on the cysts of *E. histolytica*/*E. dispar*

Product	Conditions	Concentration or CT	Inactivation of cysts (%)
Active chlorine	aqueous solution	CT: 20 mg.min/L (30°C, pH 7)/ 120 mg.min/L (pH 10)	99
Ozone	solution	0.7-1.1 mg/L	>98
Bromine	solution	CT: 15 mg.min/L (pH 4, 30°C)/40 mg.min/L (pH 10)	99.9

* CT: product of the concentration of the disinfectant multiplied by the contact time. It varies depending on water quality (variable organic load).

Effects of temperature	Other treatments
Cysts are inactivated by heating for 10 minutes at 100°C or freezing for 24 h at -0°C.	Cysts are sensitive to desiccation. They survive only 24 to 48 hours on dry soils. They can survive for between 5 and 15 days in septic tanks. There are no data on the use of ultraviolet radiation on cysts of <i>E. histolytica</i> .

Monitoring in food

In France, there are no regulations governing the detection of *Entamoeba* cysts in food matrices.

Screening in food

For protozoa, the FDA has described a method for the isolation of parasites contaminating fruit and vegetables: the products are cut and sonicated in a bath of detergent (1% SDS, 0.1% Tween 80). The lavage fluid is centrifuged and the pellet is examined directly, or after flotation in a saturated sucrose solution. Results: there are very few data. In a survey conducted in Costa Rica, *E. histolytica* cysts were identified in 2-6% of the vegetables analysed.

Screening in water and the environment

There is no standardised method for the detection of *Entamoeba* cysts in water. The literature is limited to the detection of *Entamoeba* cysts in wastewater and land-spreading water in endemic areas. The methods consist basically in concentrating particles (sedimentation, centrifugation), purifying elements using conventional parasitology techniques and examining under a microscope with or without labelling.

Recommendations to operators

- Observe good hygiene practices during processing of raw materials, use water intended for human consumption during the process.
- Kitchen staff or anyone else involved in handling foods, especially those intended to be eaten raw or partly cooked, should be made aware of the risk of faecal-oral transmission and the need to observe strict hygiene measures (washing hands thoroughly).
- This parasite should be taken into account in the hazard analysis conducted by operators concerned by foods that are immersed in or spray-irrigated by potentially contaminated water. Appropriate control measures should be taken.

Domestic hygiene

Recommendations to consumers

- Observe basic hygiene rules including thorough washing of hands, cooking utensils and work surfaces, especially before handling food and after using the toilet.
- Thoroughly wash with water intended for human consumption any foods (fruit and vegetables consumed raw) that may be contaminated by *Entamoeba* cysts.
- Cook food if it cannot be washed normally (lack of water, or water of questionable provenance) particularly in areas of high endemicity.
- In endemic areas, drink encapsulated bottled spring or mineral water. Otherwise, boil (10 min) or microfilter the water.

References and links

General references

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