INTRODUCTION

Several nonpathogenic protozoa inhabit the intestinal tract and may be identified in stool specimens sent to the clinical laboratory for ova and parasite examination [1][2]. The nonpathogenic protozoa can be divided into two groups: amebae and flagellates. Protozoa are a diverse group of unicellular eukaryotic organisms, many of which are motile. They are restricted to moist or aquatic habitats. That can be flagellates, ciliates, and amoebas (motile by means of pseudopodia).

Methods: All protozoa digest their food in stomach-like compartments called vacuoles. Some protozoa have life stages alternating between proliferative stages (e.g., trophozoites) and dormant cysts. Protozoa can reproduce by binary fission or multiple fission. Some protozoa reproduce sexually, some asexually, while some use a combination, (e.g., Coccidia). Entamoeba is a genus of Amoebozoa found as internal parasites or commensals of animals. Several species are found in humans.

Results: Entamoebahistyntica is the pathogen responsible for ‘amoebiasis’ (which includes amebic dysentery and amebic liver abscesses), while others such as Entamoeba coli and E. dispar are harmless. With the exception of Entamoebahystinovis, which lives in the mouth, and E. moshkovskii, numerous soil protozoa. They move around with whip-like tails called flagella (5-10 µm long), hair-like structures called cilia (20-30 µm long), or foot-like structures called pseudopodia (2 µm thick by 20 µm). Others do not move at all. Protozoa may absorb food via their cell membranes, some, e.g., amoebas, surround food and engulf it, and yet others have openings or ‘mouth pores’ into which they sweep food, and that engulfing of food is said to be phagocytosis. All protozoa digest their food in stomach-like compartments called vacuoles[6]. Protozoa eat about 100 to 1,000 bacteria per hour.

The pellicle is a thin layer supporting the cell membrane in various protozoa, protecting them and allowing them to retain their shape, especially during locomotion, allowing the organism to be more hydrodynamic. They vary from flexible and elastic to rigid. Although somewhat stiff, the pellicle is also flexible and allows the protist to fit into tighter spaces. Some protozoa have life stages alternating between proliferative stages (e.g., trophozoites) and dormant cysts. Protozoa can reproduce by binary fission or multiple fission. Some protozoa reproduce sexually, some asexually, while some use a combination, (e.g., Coccidia). An individual protozoan is hermaphroditic (Fig. 1).

There are three main stages to the life cycle of intestinal protozoa:
1. Trophozoite: The trophozoite stage is a fragile, vegetative stage in which the protozoan must encyst to survive in the environment.
2. Pre-Cyst: The pre-cyst stage consists of encysting trophozoites disgorging of any undigested food.
3. Cyst: The cyst stage is the infective, transmissible phase in which protozoan are resistant to harsh environmental conditions.

Fig. 1: Entamoeba Scientific Classification (http://www.bing.com/images/search?q=amoeba&FORM=HDRSC2 [7].
Entamoeba

Scientific classification

- **Domain:** Eukaryota
- **Phylum:** Amoebozoa
- **Class:** Archamoebae
- **Genus:** Entamoeba
  - *E. coli*
  - *E. dispar*
  - *E. gingivalis*
  - *E. histolytica*
  - *E. invadens*
  - *E. moshkovskii*
  - *E. hartmanni*
  - *E. polecki*
  - *E. nana*
  - *Iodamoeba butschlii*

All species are distributed worldwide. *Entamoeba* is a genus of Amoebozoa found as internal parasites or commensals of animals. In 1875, Fedor Lösch described the first known case of amoebic dysentery in St Petersburg, Russia [9]. He referred to the amoeba he observed microscopically as ‘Amoeba coli’; however, it is not clear whether he was using this as a descriptive term or intended it as a formal taxonomic name [10]. The genus *Entamoeba* was defined by Casagrandi and Barbagallo [10] for the species *Entamoeba coli*, which is known to be a commensal organism. Lösch’s organism was renamed *Entamoeba histolytica* by Fritz Schaudinn in 1903; he later died, in 1906, from a self-inflicted infection when studying this amoeba [11]. For a time during the first half of the 20th century the entire genus *Entamoeba* was transferred to *Endamoeba*, a genus of amebas infecting invertebrates about which little is known. This move was reversed by the International Commission on Zoological Nomenclature in the late 1950s, and *Entamoeba* has stayed ‘stable’ ever since.

Species

Several species are found in humans. *Entamoeba histolytica* is the pathogen responsible for ‘amoebiasis’ (which includes amoebic dysentery and amoebic liver abscesses), while others such as *Entamoeba coli* (Not to be confused with Escherichia coli) and *E. dispar* are harmless. With the exception of *Entamoeba gingivalis*, which lives in the mouth, and *E. moshkovskii*, which is frequently isolated from river and lake sediments, all *Entamoeba* species are found in the intestines of the animals they infect.

Life Cycle

*Entamoeba* cells are small, with a single nucleus and typically a single lobose pseudopod taking the form of a clear anterior bulge. They have a simple life cycle. The trophozoite (feeding-dividing form) is approximately 10-20 μm in diameter and feeds primarily on bacteria. It divides by simple binary fission to form two smaller daughter cells. Almost all species form cysts, the stage involved in transmission (the exception is *E. gingivalis*). Depending on the species, these can have one, four or eight nuclei and are variable in size; these characteristics help in species identification (Fig. 2).

Structure

*Entamoeba* cells are small, with a single nucleus and typically a single lobose pseudopod taking the form of a clear anterior bulge. They have a simple life cycle. The trophozoite (feeding-dividing form) is approximately 10-20 μm in diameter and feeds primarily on bacteria. It divides by simple binary fission to form two smaller daughter cells. Almost all species form cysts, the stage involved in transmission (the exception is *E. gingivalis*). Depending on the species, these can have one, four or eight nuclei and are variable in size; these characteristics help in species identification (Fig. 2).

### Table 1: Historical Highlights of the Genus *Entamoeba* [20].

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1875</td>
<td>Lösch demonstrated that dysentery is due an ameba, which he named <em>Amoeba coli</em>.</td>
</tr>
<tr>
<td>1891</td>
<td>Councilman and LaFleur demonstrated role of ameba in tissue invasion.</td>
</tr>
<tr>
<td>1903</td>
<td>Schaudinn renamed species to <em>Entamoeba histolytica</em>.</td>
</tr>
<tr>
<td>1925</td>
<td>Brumpt proposed two species to distinguish pathogenic and non-pathogenic infections: <em>E. dysenteriae</em> and <em>E. dispar</em>.</td>
</tr>
<tr>
<td>1957</td>
<td>Burrows classified <em>E. hartmanni</em> as a distinct species instead of the ‘small race’ of <em>E. histolytica</em> as previously advocated by Brumpt in 1926.</td>
</tr>
<tr>
<td>1973</td>
<td>Martinez-Palomó demonstrated differences in agglutination between pathogenic and non-pathogenic isolates.</td>
</tr>
<tr>
<td>1978</td>
<td>Sargeant and Williams demonstrated zymodeme differences between pathogenic and non-pathogenic isolates.</td>
</tr>
<tr>
<td>1988-93</td>
<td>Several investigators demonstrated antigenic and DNA differences between pathogenic and non-pathogenic isolates.</td>
</tr>
<tr>
<td>1993</td>
<td>Diamond and Clark proposed <em>E. dispar</em> as the name for non-pathogenic <em>E. histolytica</em>.</td>
</tr>
<tr>
<td>1997</td>
<td>WHO endorsed two species.</td>
</tr>
</tbody>
</table>

Entamoeba coli

Large cosmopolitan amoeba, commensal in man, that lives and multiplies in the large intestine. E. coli is a non-pathogenic species of Entamoeba that frequently exists as a commensal parasite in the human gastrointestinal tract. E. coli (not to be confused with the bacterium Escherichia coli) is important in medicine because it can be confused during microscopic examination of stained stool specimens with the pathogenic Entamoeba histolytica. While this differentiation is typically done by visual examination of the parasitic cysts via light microscopy, new methods using molecular biology techniques have been developed.

The presence of E. coli is not cause in and of itself to seek treatment as it is considered harmless. However, when a person becomes infected with this benign entamoeba, other pathogenic organisms may have been introduced as well, and these other pathogens might cause infection or illness.

E. Coli trophozoites can be distinguished by their wide and tapered pseudopodia. They are often mistaken for H. histolytica due to their overlap in size. The cysts are distinguished by noticing the eight nuclei found in the mature form.

Trophozoite

Size: from 15 to 50 μm; usual range, 20-30 μm.

Motility: visible only in fresh, unfixed stool specimens. The trophozoite slowly forms a pseudopod, then withdraws it and remains immobile maintaining a round shape. After a few moments, a new pseudopod forms in a new place (non-progressive) movement, without a defined direction. In some cases, it is possible to observe the simultaneous, explosive formation of 5-3 small, rounded pseudopods extend simultaneously, but even in this case the amoeba remains within the microscopic field.

Cytoplasm: in mobile trophozoites, the ectoplasm (hyaline and transparent) is clearly distinct from the endoplasm which appears unevenly granular with vacuoles often containing starch granules, bacteria, yeast but also cysts of parasites, such as Giardia intestinalis, or of other amoebae. The cytoplasm is often parasitized by Sphaerita. As a general rule, the cytoplasm does not contain red blood cells, except in the rare case of patients with intestinal hemorrhage. In these cases, Entamoeba coli trophozoites can be hematophagic.

Nucleus: 5-7 μm, often visible on fresh wet mount, although it is often impossible to clearly see the characteristics of the karyosome and peripheral chromatin. In these cases, it is appropriate to examine specimens that are fixed (with SAF) and temporarily or permanently stained.

Karyosome: large, diffuse or granular, generally eccentric. Achromatic granules (chromosomes) can often be seen around the karyosome.

Peripheral chromatin: the nuclear membrane is thick and irregular, with coarse granules of chromatin often unevenly arranged. With trichrome staining, the Entamoeba coli trophozoite (above all its nucleus) sometimes stains more intensely than trophozoites of Entamoeba histolytica or other amoebae. It is not always easy for the inexperienced microscopist to distinguish between nuclei of Entamoeba coli and Entamoeba histolytica. Therefore, for a correct diagnosis of the species, it is important to observe many trophozoites. Entamoeba coli is a non-pathogenic amoeba, so its differential diagnosis from Entamoeba histolytica is of fundamental importance to avoid patients being treated unnecessarily for amoebiasis, when the cause of their intestinal symptoms is quite different (for example, colon cancer).

Cyst

Size: 10 to 35 μm; usual range, 15-20 μm. Immature cysts are generally larger.

Shape: generally round, often oval or irregular. The cyst is surrounded by a thick double wall, hard to see in fresh specimens, that has a shell-like appearance which is clearer, more refractile and more uniform in thickness than that of Entamoeba histolytica.

Nucleus: depending on the stage of maturation, there can be from 1 to 8 nuclei. It is possible to observe cysts with 16 or 32 nuclei (supernucleate cysts).

Cysts with 1 nucleus: large nucleus, with diffuse karyosome that displays clear karyokinetic activity. In most cysts, a large (iodophilic) glycogen vacuole is present; in these cases, the nucleus is oval and confined to the periphery of the cyst. With the maturation of the cyst, the vacuole shrinks and finally disappears. Chromatoid bodies with pointed ends are visible in the space between the vacuole and the cyst wall.

Cysts with 2 nuclei: The nuclei are usually oval and located at diametrically opposite poles with respect to the vacuole. In contrast, in binucleate cysts of Entamoeba histolytica, the nuclei are generally paired.

Cysts with 4 nuclei: At this stage of maturation, the glycogen vacuole is normally absent and the nuclei are dispersed within the cyst. The nuclei can have variable sizes, some are irregular and evidently undergoing division, with coarse peripheral chromat in and a karyosome composed of granules of dispersed chromat in. Tetranucleate cysts of Entamoeba coli can be mistaken (by inexperienced microscopist) for mature cysts of Entamoeba histolytica. Therefore, it is important to remember that a tetranucleate Entamoeba coli cyst is larger (>14 μm) than a mature cyst of Entamoeba histolytica, can be variable in shape, and has nuclear peripheral chromat in and karyosome composed of irregular granules.

Cysts with 8 nuclei: These cysts are fully mature. In fresh mount preparations, the nuclei exhibit thin peripheral chromatin with a small karyosome eccentrically located. In mature cysts, which represent the infective stage, chromatoid bodies are rarely seen and the glycogen vacuole is always absent.

Entamoeba dispar

Nonpathogenic species that occurs in the large intestine of humans; formerly considered Entamoeba histolytica, Entamoeba dispar is now considered a separate species; it is nonpathogenic and is not associated with symptomatic amebiasis in humans. Morphologically it resembles Entamoeba histolytica; however, the trophozoites are never found to contain ingested red blood cells. In 1925, Brumpt formulated the theory that the difference between many asymptomatic amebic infections and those of individuals with amebic disease could be correlated with the existence of two distinct but morphologically identical species [25], namely, E. histolytica (which is capable of causing invasive disease) and E. dispar (which never causes disease). This hypothesis was dismissed at that time, but subsequently evidence which gave support to Brumpt’s findings began accumulating.

In 1993, 68 years after the original discovery of E. dispar, E. histolytica and E. dispar were formally accepted as different yet closely related species on the basis of extensive genetic, immunological and biochemical analyses.

Although E. dispar was previously considered to be nonpathogenic, E. histolytica and was regarded as a commensal species, intestinal symptoms in patients infected with this species have been reported. In a recent study from India by Panja and Kharinar [29], 68 fecal specimens in which Entamoeba species were demonstrated on microscopy were tested using PCR. Eleven patients positive for E. dispar and E. moshkovskii (in association) had mild gastrointestinal discomfort; however, the study failed to clarify whether other parasites or bacterial or viral pathogens were detected in these 11 samples.

Entamoeba dispar can produce variable focal intestinal lesions in anesthetized animals [30-32] and can destroy epithelial cell monolayers in vitro [33]. There is also some evidence that following infection with E. dispar, pathological changes may occur in some humans [34]. However Koch’s postulates have not been fulfilled [35], and no large
case-controlled studies have been undertaken to assess the true pathogenic potential of this organism.

**Entamoeba gingivalis**

*Entamoeba gingivalis* is an opportunistic protozoa reported by some to cause disease [36][37][38] and is the first amoeba in humans to be described. It is found in the mouth [39] inside the gingival pocket biofilm and near the base of the teeth. *Entamoeba gingivalis* is found in 95% of people with gum disease and rarely in people with healthy gums [40]. Cyst formation is not present; therefore transmission is direct from one person to another by kissing, or by sharing eating utensils (Fig. 5). Only the trophozoites are formed and the size is usually 20 micrometers to 150 micrometers in diameter. *Entamoeba gingivalis* have pseudopodia that allow them to move quickly and phagocytise the nucleus of polynuclear neutrophils by exocytosis or phagocytosis. They may also enter the infected periodontal crevices, besides moving, consists in feeding on the nucleus of white blood cells. The amoeba penetrates into the cytoplasm to reach the nucleus and literally suctions its contents via the negative pressure of the pseudopod. The food so gulped down is gradually digested inside the endoplasm. Phagocytosis can sometimes continue for more than 20 polynuclear neutrophil nuclei.

(Entamoeba gingivalis)

**Fig. 5: Life cycle [42]**

The trophozoite stage of *E. gingivalis* is morphologically similar to that of *E. histolytica*, and the trophozoite should be differentiated, as both can be coughed up in sputum specimens (for the latter, when present in pulmonary abscesses).

**Laboratory diagnosis**

Identification of *E. gingivalis* is made by the finding of trophozoites in scrapings of the gums and teeth. They may also be found in sputum from pulmonary abscesses.

**Trophozoite**

Size: from 5 to 12μm; usual range, 8-10μm. Trophozoites parasitized by Sphaerita may be larger and similar in size to *Entamoeba histolytica*.

Motility: similar to *Entamoeba histolytica*.

Nucleus: not visible in fresh specimens. In fixed, stained specimens, the diameter ranges from 1.5 to 3μm.

Karyosome: small and compact, in central, sub-central or eccentric position, as for *Entamoeba histolytica*.

Peripheral chromatin: small, evenly distributed granules; in some cases, granules are irregular and unevenly distributed. Wet mounts stained with MIF, Lugol’s iodine solution, or Bailenger’s stain as well as permanent stained smears reveal a nucleus that is characteristically *Entamoeba*, thereby avoiding confusion with *Endolimax nana* trophozoites which are often of the same size (Fig. 6).

Cytoplasm: may contain small vacuoles or inclusions, but in general has a uniform appearance. With trichrome staining, this trophozoite stains less intensely and appears more delicate than *Entamoeba histolytica* [46].

**Fig. 6: Entamoeba hartmanni trophozoite in stool smear. (Gomori-Wheatley stain). No distinct wall, cytoplasm is poorly coloured. Distinct nucleus showing a central karyosome and a thick chromatin**

**Fig. 7: Entamoeba hartmanni cysts (Junod technique) 2 small cysts present (7 μm) with distinct walls and vacuolized cytoplasm**

**Shape: round, sometimes oval.**

**Appearance:** clean and refractile in fresh specimens, very different from the appearance of *Endolimax nana*. 

them from the morphologically-similar trophozoites of *E. histolytica*, which may be found in sputum from pulmonary abscesses.

**Entamoeba histolytica**

*Entamoeba histolytica* was first described by Fedor Loeš in 1875 in St. Petersburg, Russia. He described intestinal amebiasis in detail, and the species name *E. histolytica* was first coined by Fritz Schaudinn in 1903 [43][8]. *Entamoeba histolytica* is the pathogenic species of *Entamoeba* that causes amebic dysentery and a wide range of other invasive diseases, including amebic liver abscess, respiratory tract infections, and cerebral and genitourinary amebiasis.

**Entamoeba invadens**

A intestinal parasite of reptiles such as snakes and lizards that causes disease similar to that caused by *Entamoeba histolytica*. Also found in turtles but does not seem to cause disease in these animals [43-45].

**Entamoeba terrapinae**

A commensal species found in turtles [43-45].

**Entamoeba hartmanni**

This small amoeba is commensal in man, colonizing the colon. It is morphologically identical to *Entamoeba histolytica*, for which it is often mistaken. Size and appearance after staining are factors in a correct diagnosis [14].

**Cyst**

Size: from 4 to 8 μm, smaller than *Entamoeba histolytica* (12-14 μm); this is a fundamental element for the differential diagnosis.
Vacuoles: small, do not stain well with Lugol's iodine solution.

Chromatoid bodies: numerous, elongated, with rounded ends, smaller than those of Entamoeba histolytica. May also be pointed or spherical. Present even in mature cysts. Given the abundance of chromatoid bodies, their presence often prevents the nuclei from being seen (Fig. 7).

Nucleus/i: The mature cyst can have from 1 to 4 nuclei, each with a diameter of 1-2 μm. However, specimens usually contain cysts with only 1 or 2 nuclei. The structure of the nucleus (karyosome and peripheral chromat) is similar to that of the trophozoite [46].

Entamoeba hartmanni is considered nonpathogenic. At one time literature referred to E. hartmanni as being a sub-species of E. histolytica. This idea has since been proved incorrect [45][46] (Fig. 8, 9). The cyst is very similar to E. histolytica but is smaller, less than 10 microns. It very often contains less than 4 nuclei but 4 nuclei are characteristic of the species. The cyst also contains chromatoidal bars, however, they are slightly smaller and more numerous. The trophozoite of E. hartmanni measuring 5-12 microns is also slightly smaller than that of E. histolytica and often contains hyperparasites. The size of the trophozoite is altered by the presence of hyperparasites. Care must be taken when differentiating the trophozoites of E. hartmanni and E. histolytica. Although E. hartmanni is considered nonpathogenic it should be considered an indicator of fecal contamination.

Laboratory diagnosis

Laboratory diagnosis is made by finding the characteristic cysts in an iodine stained, formal-ether concentration method or by detecting the characteristic trophozoites in a wet preparation or a permanent stained preparation.

Entamoeba polecki

Entamoeba polecki is a single-celled parasite that is found in intestines, mainly in pigs and monkeys [47]. Other animals that it can be found in are cattle, goats, sheep, dogs, and humans. The way humans get infected is by swallowing the parasite. The parasite is sometimes confused with Entamoeba histolytica.

Entamoeba Polecki is an intestinal protozoan which is best known for its infection of pigs and monkeys. However, E. polecki is not unique to these animals, as many human infections have been reported. Due to the rarity of this parasite, however, human clinical manifestation is not known by names other than E. polecki infection or amebiasis [47]. Entamoeba Polecki was first identified in 1912 in Czechoslovakia by Von Prowazek in the stool samples of two students from Kampuchea. Characteristic, uninucleated cysts of diameters between 14.2-15.7 microns and nuclei diameters of 3.2-4.2 microns were found repeatedly in stool samples taken from these students. Following these cases, E. Polecki was repeatedly found in pig feces, but no other human cases were reported until 1949. Most researchers believe, however, that many more human cases existed during this time and that the infections were either asymptomatic and never identified or were misdiagnosed as E. histolytica.

Although Entamoeba Polecki is rarely found in humans, it has a widespread and relatively unpredictable epidemiology. The disease is much more common in rural regions than urban areas. Most commonly, Entamoeba Polecki is associated with Papua New Guinea, where a study estimated that the prevalence was as high as 19 percent of the population. This is not surprising given the economy and culture of this country where pigs play a key role and many pigs are even allowed to live in residences. There are three other countries in which E. Polecki is endemic, including Cambodia, Venezuela, and Vietnam. Additionally, E. polecki infections have been reported in Southeast Asian refugees living in other locations, namely France, Minnesota, and Venezuela.

The trophozoites of Entamoeba Polecki are rounded with diameters of less than 10 to more than 20 micrometers, with the majority being between 12-18 micrometers. When stained, the nucleus with a small central karyosome is visible and is either seen evenly distributed or massed at one or both poles. Stained vacuoles in the trophozoites also show ingested bacteria and yeast. The peripheral chromat is most often seen as distributed granules on the nuclear membrane. The fine granules are either touching each other or having small spaces in between, but are not uniformly distributed as in the trophozoites of many similar protozoa.

The cysts of E. Polecki range in size from 9.5-17.5 micrometers, though normally are between 12-15 micrometers and are spherical or subspherical. They are almost always uninuclear and contain abundant chromatoidal material with angular or pointed, or threadlike ends. Glycogen vacuoles are also present in many of the cysts, in addition to spherical or ovoid shaped inclusion masses. As in the trophozoites, the peripheral chromat is generally distributed non-uniformly [48] (Fig. 10).

Animal reservoir

Pigs and Monkeys are the primary reservoirs for E. Polecki, though infection has also been documented in goats, sheep, cattle, and other wild ungulates.

Vector

Entamoeba Polecki has no vector.

Transmission

The exact form of E. polecki transmission is unknown, but transmission to humans via ingestion of cysts in pig or monkey feces through contaminated food is considered to be the most likely route. However, there are many reports of infected individuals that have had no contact with host animals. This indicates a strong likelihood of human-to-human transmission, especially in regions such as Papua New Guinea with high disease prevalence. The possibility of obtaining parasite from other domestic animals such as goats, sheep, cattle, and wild ungulates also exists.

Clinical presentation In humans

Infection with Entamoeba Polecki is almost always asymptomatic in humans, but debate remains about the possibility of nonspecific
Symptoms such as diarrhea, bloody stools, fever, nausea, vomiting, abdominal cramps, inspiratory restriction, and weight loss. There is conflicting evidence in the published research on this infection, though the CDC reports that any gastrointestinal symptoms must be attributed to other, non-amoebic causes.

**Incubation period**

The incubation period of Entamoeba Polecki is currently unknown. However, Entamoeba polecki greatly resembles *E. histolytica* which has an incubation period that varies between a few days and a few weeks, depending on the infective dose.

**Diagnosis**

The main method for diagnosing Entamoeba Polecki is by the identification of trophozoites in feces, utilizing preserved, stained, and microscopically examined stool specimens. This method of diagnosis can be difficult, however, given the morphologic similarities between *E. Polecki* and other intestinal amoebas such as *E. histolytica* and *E. hartmanni*. For a definitive diagnosis, electroimmunotransfer blots are frequently used to identify the antigenic structure of the parasite. Serologic tests have been shown to be insufficient in distinguishing between the three aforementioned Entamoeba species.

**Drug therapy**

*Entamoeba Polecki* has been successfully treated with the use of three antiparasitic drugs. Metronidazole, Ornidazole, and Furamide have been proven effective, though Metronidazole is the most common and debatably most effective. This drug is effective at a dose of 750 mg three times a day for 5, 7, or 10 days. Ornidazole and Furamide have been shown to treat the parasite in combination with Metronidazole, though it is still not known if these drugs are effective on their own. Interestingly, all the three commonly employed antiamoebic drugs have been ineffective in the treatment and management of this parasitic infection [50].

**Public health and prevention strategies**

Because both zoonotic and fecal-oral transmission have been suggested for *Entamoeba Polecki*, many public health and prevention strategies are possible and should be considered. Limited contact with pigs and monkeys is the most obvious. Additionally, health education strategies such as improving personal hygiene, sanitary disposal of feces, and hand washing are necessary for the prevention of transmission. Most importantly, proper cleaning, handling, and cooking of food will be essential public health interventions.

**Endolimax nana**

*Endolimax* is a genus of amoeboid [51] that are found in the intestines of various animals, including the species *E. nana* found in humans. Originally thought to be non-pathogenic, studies suggest it can cause intermittent or chronic diarrhea [52]. Additionally, it is very significant in medicine because it can provide false positives for other tests, notably the similar species *Entamoeba histolytica*, the pathogen responsible for amoebic dysentery, and because its presence indicates the host has consumed fecal material. It forms cysts with four nuclei which excyst in the body and become trophozoites. *Endolimax nana* nuclei have a large endosome somewhat off-center and small amounts of visible chromatin or none at all.

**Trophozoite**

This stage is small, measuring 6-12 µm, with an average range of 8-10 µm. Living trophozoites are sluggish and generally non-progressive. The single nucleus sometimes is visible in unstained preparations. In stained organisms, the karyosome usually is large and irregularly shaped, but occasionally it may be fragmented or placed against one side of the nuclear membrane. There is no peripheral chromatin on the nuclear membrane. The cytoplasm, which is coarsely granular and often highly vacuolated, may contain bacteria. [53](Fig. 11, 13).

**Cyst**

Cysts are small, with a spherical to ellipsoidal shape. Mature cysts contain four nuclei; immature cysts are rarely seen. These cysts may vary in size, with a usual range of 6-8 µm. In stained preparations, the nucleus has a distinct karyosome that, while not as large as that seen in the trophozoite, is still larger than the karyosome of the Entamoeba species. Peripheral chromatin is absent. Although the nuclei are not visible in unstained preparations, the karyosomes are readily apparent in iodine-stained wet mounts. The cytoplasm may contain diffuse glycogen, and chromatid bodies are absent. Occasionally, small granules or inclusions may occur in the cytoplasm [Fig. 12].

---

**Entamoeba moshkovskii**

*Entamoeba moshkovskii* cysts are morphologically indistinguishable from those of the disease-causing species *E. histolytica* and the non-pathogenic *E. dispar*. Although sporadic cases of human infection with *E. moshkovskii* have been reported, the organism is considered primarily a free-living ameba. No simple molecular detection tool is available for diagnosing *E. moshkovskii* infections [55]. *Entamoeba moshkovskii*, considered to be primarily a free-living ameba, is indistinguishable in its cyst and trophozoite forms from *E. histolytica* (the cause of invasive amebiasis) and *E. dispar* (a common noninvasive parasite), except in cases of invasive disease when *E. histolytica* trophozoites may contain ingested red blood cells. *E. moshkovskii* has so far rarely been shown to infect humans; however, the organism appears to be ubiquitous in anoxic sediments. Although the early isolations of this species were from sewage, *E. moshkovskii* can also be found in environments ranging from clean riverine sediments to brackish coastal pools [56]. *E. moshkovskii* is osmotolerant, can be cultured at room temperature, and is resistant to emetine, all characteristics that distinguish it from *E. histolytica* and *E. dispar*. Human isolates of *E. moshkovskii* to date have come from North America, Italy, South Africa, and Bangladesh, and they have never been associated with disease [57]. However, few studies have actually set out to identify such infections [16].

The structural resemblance of the apparently innocuous *E. moshkovskii* to the disease-causing *E. histolytica* makes differentiating the two species important. In the clinical setting, for
example, an *E. moshkovskii*-infected patient could be diagnosed as infected with *E. histolytica* and be treated unnecessarily with antiamoebic chemotherapy. Most studies that have investigated the prevalence of *E. histolytica* and *E. dispar* have not considered the possible presence of *E. moshkovskii*, partly because of a lack of tools to detect *E. moshkovskii* other than cultivation, which is labor-intensive, not always successful, and problematic in the case of mixed infections.

several important findings. The overall *E. moshkovskii* prevalence (21%) suggests that this infection is common among these children. *E. dispar*-infected children were almost twice as likely to have a mixed infection with *E. moshkovskii* (35%) compared to those with (18%) or without *E. histolytica* (18%) infections. None of the six children with *E. moshkovskii* mono- or mixed infections had diarrhea or dysentery, which suggests that *E. moshkovskii* is a noninvasive parasite. The high prevalence of *E. moshkovskii* infection may have been unnoticed over the years because most such infections (74%) were mixed infections with *E. histolytica*, *E. dispar*, or both. Previous attempts to identify human *E. moshkovskii* infections [58][59] may have failed because the human intestinal flora was unsuitable for cultivation at room temperature.

The high prevalence of *E. moshkovskii* shown in this study population indicates that perhaps humans are a true host for this putatively free-living ameba and are not just transiently infected. This prevalence may also explain some of the microscopy-positive/antigen-negative results obtained when using the *Entamoeba* test kit [60]. Epidemiologic studies of *E. histolytica* infection should include tools to diagnose all three of these species individually, simultaneously, and accurately, and the prevalence of *E. moshkovskii* infection in other regions of the world should be investigated.

**Iodamoeba bütschlii**

It gets its name from its appearance when stained with iodine[61]. Named for Otto Bütschli by Prowazek in 1912 [62], *Iodamoeba bütschlii* is a nonpathogenic parasitic ameba, commonly found in the large intestines of people, pigs and other mammals.

The distribution of *I. bütschlii* is worldwide. Most likely to be the original host, pigs are often targeted with *I. bütschlii*. *I. bütschlii* is identified as a non-pathogenic parasite. Often, this parasite is mistaken as a pathogenic parasite because non-pathogenic parasites have the same characteristics. In terms of illnesses, humans have a low prevalence of *I. bütschlii* (4-8%). *I. bütschlii* is an indicator of oral-fecal contamination and humans may experience diarrhea [63][64].

**Trophozoite**

The trophozoites are 9–14 micrometres in diameter. Trophozoites are one of the two forms of *I. bütschlii*. This form has a pseudopodia for locomotion. The pseudopodia is short and blunt. It moves in a slow manner. The trophozoite has a single nucleus, prominent for nuclear endosome and many cytoplasmic vacuoles. The ectoplasm and the granular endoplasm are often hard to distinguish. The nucleus is fairly large and vesicular, containing a large endosome, surrounded by light staining granules about midway between it and the nuclear membrane. Achromatic strands stretch between the endosome and nuclear membrane without any peripheral granules.

Food vacuoles are commonly filled with bacteria and yeast. Trophozoites are often identified by a stool smear, found in loose stools [65](Fig.14)

**Cyst**

The cysts are 8–10 micrometres in diameter, with a thick wall and a large glycogenvacuole that stains darkly with iodine. Usually harmless, it may cause amebiasis in immunologically compromised individuals. As the second form of *I. bütschlii*, cysts have an oval shaped- single nucleus with a prominent nuclear endosome. This form is also large, single, glycogen-filled vacuole called iodophilic vacuole (glycogen stains with iodine). Cysts are the infective stage of *I. bütschlii*. Unlike trophozoites, cysts are often found in formed stools [65] Fig.15, 16.

Fig. 14: *Iodamoeba bütschlii* trophozoite in a stool smear with 2 active trophozoites emitting a rounded refractive pseudopod. They can produce several pseudopods at the same time limiting their movement. A concentration technique will confirm that they are in fact *I. bütschlii* trophozoites.

Fig. 15: *Iodamoeba bütschlii* cysts, easy to diagnose due to their size (13 m), the presence of a large well defined vacuole in the cytoplasm. By refoucus you can see the karyosome which appears like a large black spot. These elements allow the diagnosis to be made.

Fig. 16: *Cyst of Iodamoeba bütschlii* stained with trichrome. Note the glycogen vacuole (arrow)

**Treatment**

In a research study, amebas were seen in stool samples of a patient and identified as *I. bütschlii*. The patient was treated with dehydroemetine and chloroquine. After treatment they noticed the patient's complement fixation titer decreased to 1:2 in a serum sample, obtained two months later.

**The nonpathogenic flagellates include**

1. *Trichomonas hominis*
2. *Chilomastix mesnili*
3. *Trichomonas tenax*

**Trichomonas hominis**

Pentatrichomonas hominis (formally known as *Trichomonas hominis*). This species is also referred to as *Trichomonas hominis* and has a variable number of anterior flagella (three to five) but typically five. The posteria flagella is attached by an undulating membrane which runs the full length of the cell. A relatively common flagellate which may be overlooked or unrecognized during examination of fecal specimens for parasites. This organism is found worldwide in both warm and temperate climates. Trophozoites are about 5 to 15 µm by 7 to 10 µm in size and are roughly pyriform in shape [66](Fig.17).

Its detection in fecal specimens does not seem to be related to gastrointestinal illness although it is often recovered from diarrheic stools. *Trichomonas hominis* is considered to be nonpathogenic. It has a relatively wide host range and is generally a harmless commensal found in the caecum and colon of man, other primates, dogs and cats. It is the least common of the 3 species inhabiting humans and is generally present in less than 2% of the population although in many developing countries the prevalence is much higher (e.g. Mexico 32%). It is said to be less common in temperate climates but increased prevalence is usually directly associated with
poor hygiene since the parasite is transmitted by the oral-faecal route via contaminated food, water and flies etc. Infections with T. hominis are easily distinguished from the other two species since there is a strict habitat restriction and this species will not survive in either the oral cavity or the genitourinary tract.

Fig. 17: Pentatrichomonas hominis, a nonpathogenic flagellate [67]

Although not proved to be pathogenic infections are often associated with other protozoal gut parasites such as Entamoeba histolytica but their presence is probably coincidental and secondary to the primary pathogen.

Diagnosis

In freshly passed stool they may be recognized by motility provided by the characteristic movement of flagella and rhythmic beating of the undulating membrane. These rather small organisms are difficult to see in the fresh specimen and are easily overlooked in stained preparations [68]. Both Trichomonas hominis and Trichomonas vaginalis are site specific in that neither can survive in the other's environment. Attempts to transplant T. hominis into the vaginal were unsuccessful [69][70].

Diagnostic and infective stage

Trophozoite

Size of trophozoite: 8 - 14 microns. Non-pathogenic Pyriform shaped body with an undulating membrane extending the entire length of the body. T. hominis has one lancet shaped very granular anterior nuclei. T. hominis is not to be confused with the vaginal, T. vaginalis, which is sometimes found as a contaminant from the vagina [71]. Prevention is by interrupting transmission which is accomplished though increased hygiene and improved sanitary conditions.

Treatment is not warranted.

Chilomastix mesnili

Chilomastix mesnili is a non-parasitic [72] member of primate gastrointestinal microflora, commonly associated with but not causing parasitic infections. It is found in about 3.5% of the population in the United States. In addition to humans, Chilomastix is found in chimpanzees, orangutans, monkeys, and pigs (Fig. 18). It lives in the cecum and colon. C. mesnili has a similar life style to Giardia lamblia. Although Chilomastix mesnili is considered non-pathogenic, it often occurs with other parasite infections. C. mesnili may be confused with other pathogenic species during diagnosis. It can create a false positive which would result in unnecessary treatment or a false negative which would hold necessary treatment.

Chilomastix mesnili trophozoites, trichrome stain.

Collapse This Group

Chilomastix mesnili cysts, trichrome stain.

Collapse This Group

Chilomastix mesnili cysts in wet mounts.

Fig. 18: Diagnosis (72).

Trichomonas tenax

Trichomonas tenax, or oral trichomonas, is a species of trichomonas which is found in the oral cavity of humans, dogs and cats. The usual hygiene is generally not sufficient to eliminate the parasite, hence its name, Latin for "tenacious". It is a parasite sometimes called unimportant although frequently encountered in periodontal infections, affecting more than 50% of the population in some area, but it is most often ignored. However, it remains absent from commensal gingival biofilm said appearing healthy. Its presence in necrotizing ulcerative gingivitis. The typical Trichomonas tenax trophozoite is described as being oval to pear shaped and measuring 5 to 14 μm long, with an average length of 6 to 9 μm. The single ovoid vesicular nucleus is filled with several chromatin granules and is usually located in the center anterior portion of the organism.

The T. tenax trophozoite is equipped with five flagella, all of which originate at the anterior end. Four of the flagella extend anteriorly and one extends posteriorly (Fig. 19). An undulating membrane that extends two thirds of the body length and its accompanying costa typically lie next to the posterior flagellum. A thick axostyl runs along the entire body length, curving around the nucleus, and extends posteriorly beyond the body of the organism. A small anterior cytosome is located next to the axostyle opposite the undulating membrane [73].
Trichomonas tenax trophozoites survive in the body as mouth scavengers that feed primarily on local microorganisms. Located in the tartar between the teeth, tonsillar crypts, pyorrheal pockets, and gingival margin around the gums, T. tenax trophozoites multiply by longitudinal binary fission. These trophozoites are unable to survive the digestive process. The typical Trichomonas tenax infection does not produce any notable symptoms.

On a rare occasion, T. tenax has been known to invade the respiratory tract, but this appears to have mainly occurred in patients with underlying thoracic or lung abscesses of pleural exudates [74].

**Laboratory diagnosis**

The specimen of choice for diagnosing *Trichomonas tenax* trophozoite is mouth scrapings. Microscopic examination of tonsillar crypts and pyorrheal pockets of patients suffering from *T. tenax* infections often yields the typical trophozoites. Tartar between the teeth and the gingival margin of the gums are the primary areas of the mouth that may also potentially harbor this organism. *T. tenax* may also be cultured onto appropriate media (Fig. 20).

The nonpathogenic amebae and flagellates listed above are enteric organisms, with the exception of *E. gingivalis* and *T. tenax* which is not an enteric organism; it is encountered around teeth (pyorrhea alveolaris), in tonsillar crypts, and in vaginal and cervical smears of women with intrauterine devices. *Entamoeba gingivalis* and *Trichomonas tenax* were the first commensal found in human oral cavity, they occur only as a trophozoit, and these are found in gingival tissues, particularly in suppurative and inflammatory processes, due to there being preference for anaerobic environments [73]. Some authors believe that these commensal could be opportunistic, that these, capable of proliferating in a gingival environment modified by periodontal and gingivitis disease [75].

The trophozoites of *Entamoeba gingivalis* and *T. tenax* are most probably transmitted from person to person by close contact since they exhibit only slight resistance to the environment. Kissing may play a role in transmission, but direct passage by many ways such as mutual usage of cups, spoon, fork, and subjects contaminated by the mouth that may also potentially harbor this organism. *T. tenax* may also be cultured onto appropriate media (Fig. 20).

The nonpathogenic protozoa listed above can be identified based on morphologic features, with the exception of *E. dispar* and *E. moshkovskii*, which cannot be morphologically distinguished from one another or from *E. histolytica*. Molecular, genetic, immunologic, clinical and other criteria can be used to differentiate the species, however. In most cases, a clinical diagnosis of amoebiasis can be confirmed and usually depends on the visualization of parasites by light microscopy of a wet smear or stained specimens.

Multiple samples often have to be requested and examined, and the presence of cysts of different species of *Entamoeba, Iodamoeba, or Endolimax* can make the diagnosis even more difficult [77,78]. There is a need for simpler and better tools suitable for identification of these amoebae in clinical specimens, not only for diagnostic purposes and patient care management, where *E. moshkovskii*/*E. dispar*-infected patients could be treated unnecessarily with antiamoebic chemotherapy, but also for a better understanding of the epidemiology of these parasites in the human population.

Although several PCR methods have been applied to the detection of *E. histolytica* and *E. dispar* in stool samples [79], there is so far only one report of PCR being used for the identification of *E. moshkovskii* in stool samples [80]. The sensitivity of these methods for detecting *E. moshkovskii* has been shown to be higher than that of microscopy [81-83]. Antibody-based methods have also been developed for the differentiation of *E. histolytica* and *E. dispar* in stool samples [84] but so far not for *E. moshkovskii*. The application of this new PCR assay as an alternative tool in routine diagnosis and in epidemiological studies of amoebiasis. It is hoped that this will provide better epidemiological data and a greater understanding of infections with these three amoebae in humans.

Antigen detection using fecal ELISA is another diagnostic tool [85]. However, the sensitivity of the fecal antigen test is about 100 times less than that of PCR, and in addition, several studies have highlighted low specificity because of cross-reaction with other *Entamoeba* species. The development of molecular tools, including PCR and real-time PCR, to detect *E. histolytica*, *E. dispar*, and *E. moshkovskii* DNA in stool or liver abscess samples has led to major advances in making an accurate diagnosis during in tropical and subtropical countries where amoebiasis is endemic, the standard clinical approach is to treat all asymptomatic individuals with cysts in feces with an antiprotozoal agent. This approach to treatment causes indiscriminate use of antiamoebic agents and has led to increased MICs of these therapeutic agents against *E. histolytica*, with a potential for resistant strains to appear [85,22]. These considerations suggest that positive fecal samples should be confirmed with reliable tests prior to initiation of therapy.

Such studies will then lead to a better understanding of the public health problem and measures to control the disease.

---

**Table 2: Non-Pathogenic Amoeba Several species of protozoans may be mistaken for Entamoeba histolytica. Care must be taken to correctly identify the infection so that the correct treatment can be administered or other infectious agents sought after.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entamoeba dispar</td>
<td>Morphologically identical to <em>E. histolytica</em>. It must be separated by isoenzymatic, immunologic or molecular analyses.</td>
</tr>
<tr>
<td>Entamoeba hartmanni</td>
<td>Some consider this a separate species. It differs from <em>E. histolytica</em> by being smaller in size.</td>
</tr>
<tr>
<td>Entamoeba coli</td>
<td>Distinguished from <em>E. histolytica</em> by having an eccentric endosone, and mature cysts with 8 nuclei. If chromatoidal bodies are present, they have splintered ends, rather than rounded as in <em>E. histolytica</em>.</td>
</tr>
<tr>
<td>Endolimax nana</td>
<td>This is a very small amoeba (6-15um) with a large, eccentric endosome and thin nuclear envelope. Mature cysts contain 4 nuclei.</td>
</tr>
<tr>
<td>Iodamoeba butschli</td>
<td>Both the trophozoite and cyst have one nucleus with a large endosome. The cyst contains a large glycogen vacuole that stains darkly with iodine.</td>
</tr>
</tbody>
</table>
Table 3: Non-Pathogenic Flagellates Several species of protozoa may be mistaken for Giardia lamblia. Care must be taken to correctly identify the infection so that the correct treatment can be administered or other infectious agents sought after.

| Chilomastix mesnili | This species is approximately the same size as C. lamblia but has only one nucleus in both the cyst and trophozoite stage. The trophozoite has 3 anterior flagella and a prominent cystosome. The cyst is lemon-shaped. |
| Retortamonas intestinalis* | Similar to Chilomastix mesnili, but 1/3 the size and with one anterior and one posterior flagellum. Cysts are also somewhat lemon-shaped. |

*Retortamonas is a genus of flagellate protozoa that live in the intestines of various animals

CONFLICT OF INTERESTS
Declared None

REFERENCES

8. Lósch F. Massenhafte Entwicklung von Amöben im Dickdarm. Virchow’s Archiv 1875;65:196-211.
29. Rubén Darío Heredia, Jairo Andrés Fonseca, Myriam Consuelo López. Entamoeba moshkovskii perspectives of a new agent to be considered in the diagnosis of amebiasis (Khairnar and Parija proposed a nested multiplex PCR in which the first PCR amplifies a genus specific sequence and the second PCR is a multiplex 2012;12(3):139–45.
35. Koch’s postulates are four criteria designed in the 1880’s to establish a causal relationship between a causative microbe and a disease; Available from: URL: https://www.boundless.com/microbiology/principles-of-epidemiology/koch-s-postulates/.