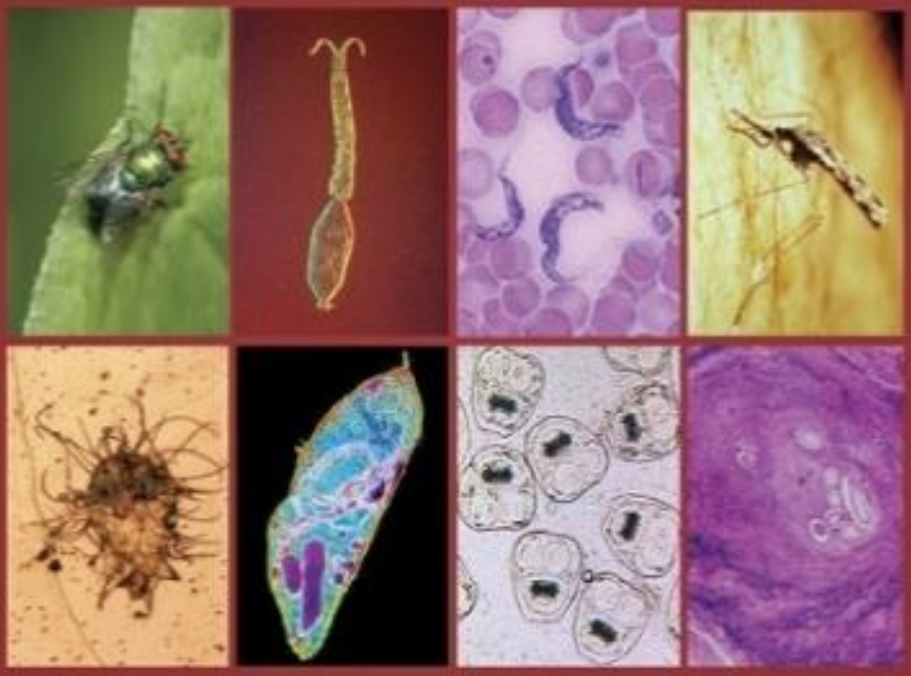
EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

E

MEDICAL ENTOMOLOGY & PARASITOLOGY



ISSN
2090-0783

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

Citation: *Egypt. Acad. J. Biolog. Sci. (E-Medical Entom. & Parasitology Vol.14(1) pp **87-100**(2022)*
DOI: 10.21608/EAJBSE.2022.234564

Egypt. Acad. J. Biology. Sci., 14 (1):87–100 (2022)

Egyptian Academic Journal of Biological Sciences
E. Medical Entomology. & Parasitology

ISSN: 2090–0783
www.eajbse.journals.ekb.eg

Virulence of *Entamoeba histolytica*

Al-Ahzzaa, Ghaida Rashid
Princess Nourh Bint Abdulrahman University, Science College, Biology department
E-mail: alhazzaaghaida@gmail.com

REVIEW INFO
Review History
Received:30/3/2022
Accepted:28/4/2022
Available:2/5/2022

Keywords:
Virulence, *Entamoeba histolytic*, Ca2+, Ach, EhGEF.

ABSTRACT

Entamoeba histolytica is an invasive protozoan, *E. histolytica* can adapt to the host's gut environment due to the virulence of the parasite. Ca2+, Acetylcholine (ACh), Fe, and EhRho1 are believed to be important for parasite motility and adhesion. Cytolysis, phagocytosis, and microcytosis cause parasitic invasion. Amebiasis is treated with metronidazole (MTZ), although strains resistant to metronidazole have emerged. With the help of studying the virulence of the parasite, it was possible to search for other drugs.
This review aims to present the key enzymes and the latest studies on promising drugs.

INTRODUCTION

Amoebiasis is an infectious parasitic disease in humans and one of the deadliest parasites in the world with a strong pathogenic possibility (Betanzos *et al.*, 2019). The life cycle of *E.histolytica* begins when infection cysts are ingested by swallowing polluted water or food and travel through the digestive system until they reach the large intestine, Infection occurs indirectly there are multiple stages and mechanisms for the onset of the disease (Kantor *et al.*, 2018). *E.histolytica* can invade and destroy human tissues, using many molecules and biological properties related to virulence. The amoeba utilizes three main virulence factors: Gal/GalNAC lectin, amebapore, and proteases. scientists work hard to understand the pathogenesis of the disease, however, the details about the sub- pathways involved in tissue invasion and damage are unclear (Babuta et al., 2020). An understanding of the virulence of the parasites and the signals that lead to invasion is needed to design a better treatment. several virulence factors have been identified, and an unclear association between parasite genotype and invasive disease has been observed (Debnath *et al.*, 2019a). It is believed that the gut environment and the parasite's genotype, along with the host's genotype, all interact to create an environment conducive to invasion by *E. histolytica* ("Hunter's Tropical Medicine and Emerging Infectious Diseases," 2020).

1. Morphology

E. histolytica occurs in four forms, trophozoite, precyclic, cystic, and metacyst stages. (Chakraborty, 2004; Paniker's, 2018).

1.1. The Trophozoite (Fig 1):

It's the vegetative feeding phase of the parasite (Chakraborty, 2004). It is a facultative arrow that metabolizes glucose as the main energy source. The trophozoite is measured in 20-30 μm. In the cytoplasm, there's a central nucleus called the karyosome. Some soluble lysosomes and organelles perform the same function as mitochondria called mitosomes. Researchers thought that mitosomes are debris resulting from the breakdown of the mitochondria organelles (Despommier *et al.*, 2017). Endoplasm contains food vacuoles containing erythrocytes sometimes leukocytes and tissue residues that the parasite swallowed from the host's body (Paniker's, 2018).

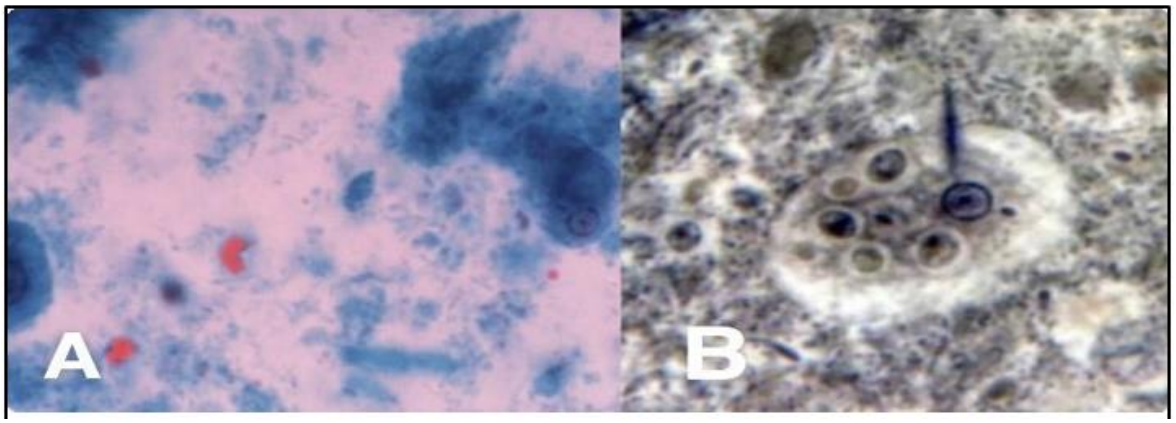


Fig. 1: **(A)** Trophozoite contains one nucleus and dimeter is 10-60 μm
(B) Trophozoite of *E. histolytica* in stool of patient suffering from amoebic (Guerrant *et al.*, 2011)

1.2. Cystic Stage (Fig 2):

The diameter of the cyst is 10-15 μm. *Entamoeba histolytica* infection occurs when ingesting cysts (Guerrant *et al.*, 2011). It has a cyst wall to protect it from the harsh environment, it's resistant to dehydration, even some chemicals such as (chlorinated compounds, fluorides) and can live in water for a month, while those in feces can live in drylands for more than 12 days, and can withstand temperatures of up to 50 C (Bogitsh *et al.*, 2012). It contains four or fewer nuclei Initially, it has one nucleus in the precyst stage, then it multiplies by binary fission and becomes two nuclei, then four nuclei, and the adult cyst contains four nuclei. Immature cysts may supplement their growth outside when they're excreted through the feces (Chakratborty, 2004; Guerrant *et al.*, 2011; Bogitsh *et al.*, 2012).

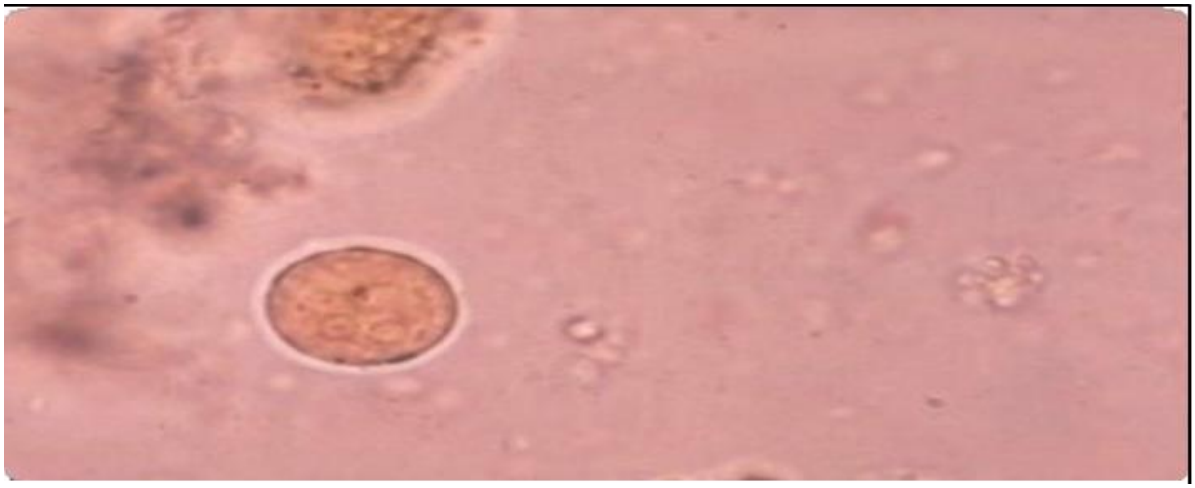


Fig2: Cysts (the infer stage) contain four nuclei, the shape of the cyst 10-15 μm (Guerrant et al., 2011

````
````

2. Life Cycle (Fig 3):

Entamoeba histolytica passes its life cycle in one host, humans. The infection is transmitted by swallowing cysts in contaminated food or water, which pass through the stomach without damage due to the cyst wall, is resistant to gastric juice (Paniker's 2018), it reaches the ileum where its exception occurs, the cyst wall shattered due of the alkaline medium of the caecum or lower part of the ileum (Guerrant *et al.*, 2011). This results in the metacystic where the mitotic divided produces it causes the nucleus division first, followed by the cytoplasm then there will be eight small amoebulae. Each amoebulae can evolve into trophozoites. There are small size trophozoites that feed on bacteria. The large ones, feed on erythrocytes and attack the epithelial cells of the mucosal epithelium of the large intestine, causing amoebiasis. Some develop into cysts in the bowel lumen that passed into the feces to repeat the cycle (Baker 2017; Chakratborty 2004; Bogitsh ., *et al.*, 2012).

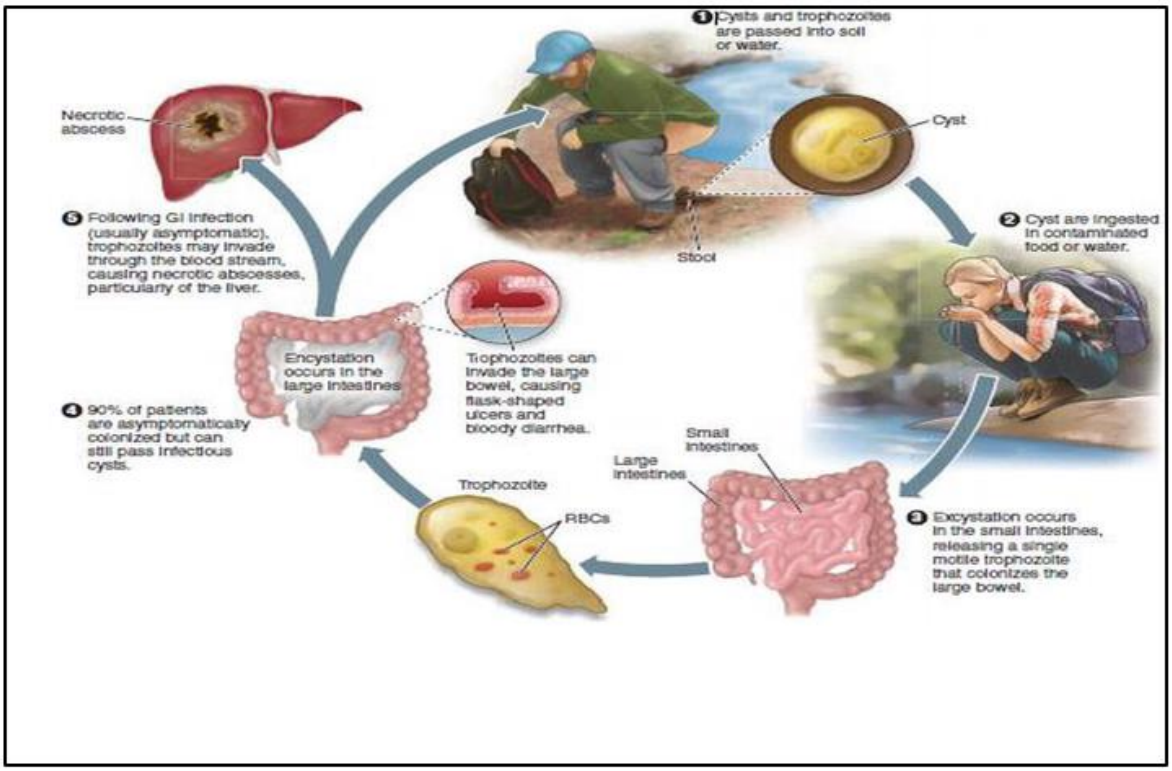


Fig3: The life cycle of *Entamoeba histolytica*

3. Amebic Dysentery

In most injuries, the inflammation resides on the mucous membrane, where the process of repairing and repairing the damaged tissue equals. It affects the gut and also the liver, lungs, and brain (Chakraborty, 2004; Pritt & Graham Clark, 2008). the *E. histolytica* is nourished by cellular debris and bacteria. The injury occurs when cysteine Proteases from *E. histolytica* digest the Muc2 mucin that surrounds the inner and outer layers and eliminates collagen and fibronectin, causing the trophozoite to invade the deep layers of the gut. normally this process is following the regeneration of damaged tissue of the intestinal wall for a period, leading to the deposition of fibrous connective tissue (Nozaki & Bhattacharya, 2015; Rogier et al., 2014; Song *et al.*, 2020). Severe ulceration follows a bacterial infection, which increases tissue breakdown, this infection is called amoebic colitis (Fig 4) (A. Singh *et al.*, 2009). Due to the high activity and high mobility of trophozoites,

when they traverse the epithelium and invade the tissues, they can reach the liver through the lymphatic and mesenteric veins to the hepatic portal vein, resulting in amoebic hepatitis and amoebic liver abscess (Fig 5) (Abubakar et al., 2020), Usually, this leads to a single abscess in the right upper lobe. The parasite multiplies rapidly in the liver, causing blockage of the blood circulation leading to ischemic necrosis of the surrounding liver cells, containing the formation of small abscesses, it becomes one large abscess when the small abscesses merge (Bogitsh *et al.*, 2012; Samie *et al.*, 2012). The abscess contains a pus-like (not pus) mixture of liver tissue and blood and appears brow and is called anchovy-sauce pus. Liver abscesses are grave given the fact that they release toxic substances due to ulceration (Chakraborty, 2004). There is a rare infection of the lungs and brain, and pulmonary amebiasis usually occurs as a complication of amoebic abscess and adheres to the diaphragm when trophozoite invades the portal circulation to the pulmonary capillaries and the appearance of abscesses like those in the liver. Cerebral amebiasis is an infection caused by a liver or lung abscess through the spread of trophozoites in the blood (Chakraborty, 2004; Samie *et al.*, 2012).

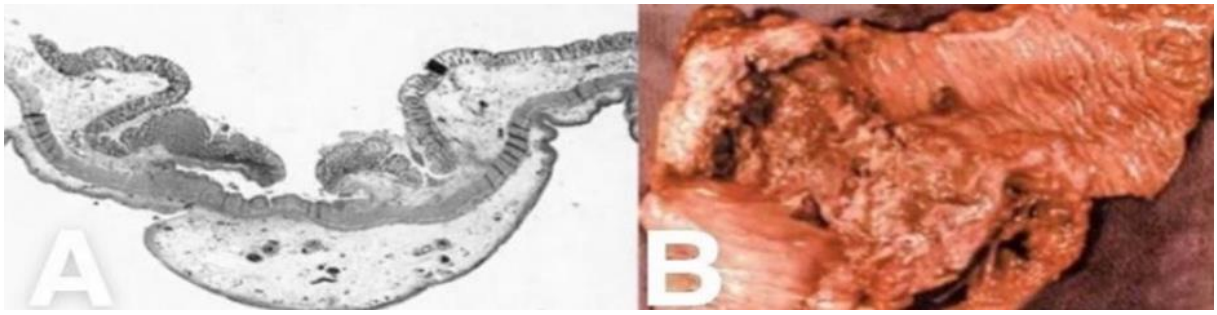


Fig 4: **(A)** Section of the human colon showing chronic amoebic ulcer (Bogitsh et al., 2012). **(B)** a portion of the transverse colon showing extensive ulceration due to intestinal infection with *E. histolytica* (Baker 2017).

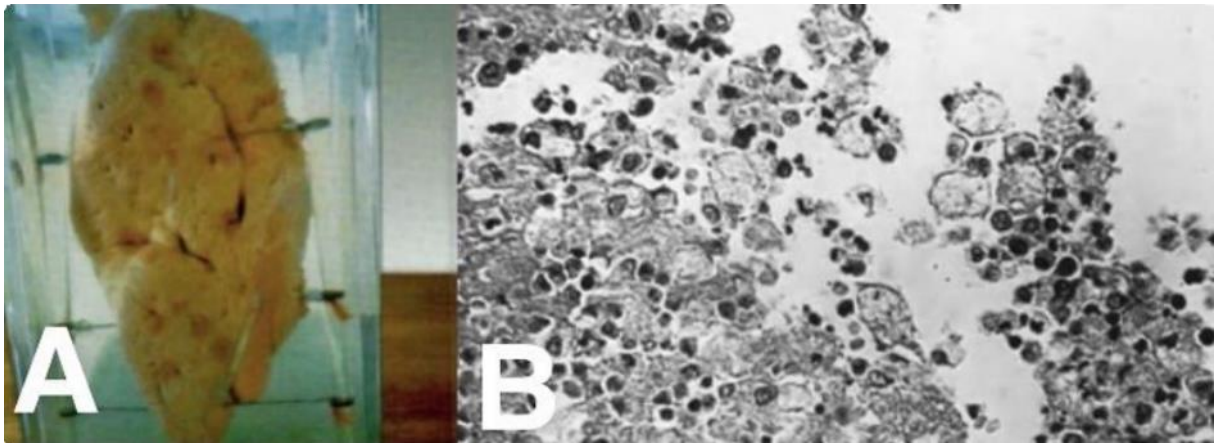


Fig 5: (A) Abscess in the human liver due to *Entamoeba histolytica*.
(B) Specimen showing amebic liver abscess (Paniker's 2018).

4. Virulence Factors of *E.histolytica*

4.1. Iron (Fe) Virulence Factors of *E.histolytica*

Iron is dependent on many organisms, including parasites and their hosts. *E. histolytica* depends on iron for metabolism, reproduction, and survival in the host body. Iron is toxic and insoluble in the host cells mammalian host cells avoid iron toxicity by isolating certain proteins such as transferrin serum and mucosal lactoferrin as part of the prosthetic head group in hemoglobin (Hb) and cytochromes in iron storage protein ferritin (ft) (Hernandez-Flores

et al., 2016). These strategies prevent the parasite from growing in the host's body, since the parasite needs iron to survive and complete its life cycle, and to cause disease the host must have access to a cation at the site of infection, the parasite evolves special mechanisms to gain iron, namely reduction of the Fe + 3 to Fe + 2 complex by ferric reductase, which releases iron, and also generates specific receptors for iron-containing host protein and the secretion of a protease .which separates iron from the protein cleavage (Gastelum-Martínez et al., 2018). E. histolytica can harness hemoglobin as a source of iron and externalize this protein across the cell membrane of erythrocytes through hemolysins and phospholipases (Cruz-Castañeda et al., 2009). Also, trophozoites have evolved two blood-binding proteins, Ehhmbp45 "EHI_096540" and Ehhmbp26 "EHI_022250." These proteins are expressed by iron deficiency and can bind to the protoporphyrin ring in heme, suggesting that they have the same function as homophones. The mechanisms of hemoglobin endocytosis to obtain iron in *E.histolytica* are not yet clear, however, *E.histolytica* can phagocytose red blood cells and obtain hemoglobin through hemoglobinase, which has optimal protein vitality at acidic pH (Cruz-Castañeda et al., 2009, 2011). *E.histolytica* has evolved many strategies to exploit host resources and evade its defenses (Anaya-Velázquez & Padilla-Vaca, 2011). Parasite virulence depends on gene expression and variables in the host environment, including iron. Iron regulation was studied in *E.histolytica* by assessing different gene expressions in the medium, either deficient or excessive in iron (J. Lee et al., 2008). The mRNA differential display technique has been used in the study of cultures grown under iron-deficiency conditions. The researchers identified several genes involved in translation including those that encode the EF-1α surveillance factor and five ribosomal proteins. These data indicate that low levels of aberration increase the need for translational mechanisms. what's more, a gene was found that codes for CP (Hernández-Cuevas et al., 2014) . Similarly, under iron-restricted conditions gene expression was significantly increased in 6 of the CPs (EhCP1 to EhCP6) (Gastelum-Martínez et al., 2018). CPs were implicated in *E. histolytica* adherence to enterocytes, which are important for host defense suppression. Cruz-Castaneda identified two types of proteins associated with *E.histolytica*, Hb-binding proteins of 45 and 26kDa (Ehhmbp 45 and Ehhmbp26 respectively). Both are involved in extracting iron from the blood. It is possible that they are hemophores that scavenge the heme from Hb or other heme proteins and can therefore be considered virulence factors of the parasite (Cruz-Castañeda et al., 2009; Gastelum-Martínez et al., 2018).

4.2. EhGEF virulence factors of *E.histolytica:*

GEF proteins help and facilitate the associated protein activation of Rho (small GTPase) by exchanging GDP with GTP. Direct activation of small GTPases with their master regulators (GEF) mediates many essential cellular activities in high eukaryotic cells, including cell morphology, actin dynamics, gene transcription, cell cycle progression, apoptosis, and malignancy progression in human cells (Toma-Fukai & Shimizu, 2019). Trophozoites interact with various host cells and tissues during invasive processes and metastasis (Cui et al., 2019), Generate signals for phagocytosis and trogocytosis to begin along with motility. Small GTPases are crucial regulators of cytoskeletal dynamics, hence they play a significant function. The Rho GTPases family plays a key role in actin dynamics by modulating the activity of actin-regulatory proteins (Mao & Finnemann, 2015; Xu et al., 2018), since GEFs act as the main regulator of the activity of Rho routines act. A study through structural bioinformatics analysis of EhGEF revealed that it is a PT-barrel structure capable of binding GTP and is not like any Dbl-family GEF structure the peptide sequence also revealed that EhGEF differs from the GEF family in length and sequence. Secondary structure predictions from

different software revealed that EhGEF is an essential part of α helices and β-sheets, whereas the conventional Dbl-family GEF structures are formed exclusively of α helices. The main function of GEF is to bind exchanges with GDP in the Rho protein pocket with GTP (Bharadwaj et al., 2017). Neither the T34N nor the EhRho1 mutant Q63L support this exchange due to a mutation in their regulatory domain (Bharadwaj et al., 2018). MANT-GTP binding was tested in a study and the results showed that EhGEF showed almost no activity towards EhRab1a, while EhRho1 was well activated. However, EhGEF showed a slow association with GTP, which was confirmed by the tryptophan quenching assay. However, MANT-GTP in a reaction containing EhGEF and EhRho1 showed a significant increase in rate and density, suggesting that EhGEF may swap nucleotides on EhRho1 by yet unknown mechanisms (Bharadwaj et al., 2021; Bosch et al., 2012). A study showed that EhGEF is one of the most important activators and specific regulators of EhRho1 in vivo. Sequence analysis showed that EhRho1 is identical to human HsRhoA except that the amoeba molecule lacks a distinct and conserved feature of the Rho GTPases "Rho insert domain", which is required for activation of the downstream Rho-associated kinase. However, both human and amoebic molecules appear to be similar in that they function in which overexpression of EhRho1 can be generating stress fibers in mammals (Bharadwaj et al., 2021; Hall et al., 2006). The GDP-bound form of EhRho1 lies in the cytosol, while the active form (GTP-bound) transports toward the membrane and attaches to it through the prenylation domain. Like EhRh1, EhGEF localization has also been observed in the cytosol and membrane of trophozoites. EhGEF can bind to the P3 "Ptflns (3,4,5)" in the membrane through the PH domain and activate EhRho1 in its way. participation in Rho-GEF in endocytosis has been well studied. The Rho-GEF has been found with Fc receptors during phagocytosis (Buchsbaum, 2007; García-Mata & Burridge, 2007). Therefore, a study examined the participation of EhRho1 in phagocytosis, they found EhGEF in phagocytic cups next to EhRho1 and a reduced formation of phagocytic cups on their down-regulation of expression. The threshold concentration of active EhRho1 (GTP bound) is Required at the RBC attachment site, for the initiation and progression of the phagocytic cup. It has been observed that phagocytosis proceeds slowly in those trophozoites down-regulated for EhGEF expression where there is sufficient EhGEF down-regulation by antisense RNA, thereby prolonging the time it takes for EhRho1 activity to reach the critical level reached on spot. Therefore, the rate of formation and development of the phagocytic cup may be directly proportional to the concentration of some key molecules, such as EhRho1 and its regulators on the phagocytic cups (Dong et al., 2010; Hall et al., 2006; W. L. Lee et al., 2007)

4.3. Ca2+ virulence factors of *E.histolytica*:

E.histolytica contains ironically released Ca2+ totaling 70% Ca2+ which can be divided into two parts:

The first part is impelled by the second messenger inositol 1,4,5-triphosphate (Ins (1,4,5) P3), which releases endogenous Ca2+ from the endoplasmic reticulum-like structures. The second part is sensible to ins (1,3,4, 5) P4 (Babuta et al., 2020). Although these two messengers' affairs in two disparate Ca2+ stores, it is unclear whether there is a connection between them in this organism *E.histolytica* cipher calpain-like protein, as well as many nuclear components that require Ca2+, including Ca2+-dependent ATPase/ADPase, Ca2+-dependent thiamine pyrophosphatase, and acid phosphatase. Calpain-like protein is expected to be interrelated with apoptosis in parasites, as its level increases during programmed cell death. It is also found in the cytoplasm and near the cell nucleus (Domínguez-Fernández et al., 2018; Monroy et al., 2015), The inner layer of cytoplasmic vacuoles, which may or may not be phagocytic, contains a small number of nucleotide enzymes. unclear whether these enzymes are into in calcium homeostasis in this parasite, genomic analysis identified 27

multi-EF-hand-containing CaBPs in E. histolytica (Bhattacharya et al., 2006). Various of these proteins are thought to be Ca2+ buffers and are accordingly involved in governing Ca2+ concentration in different cell compartments. The first step: The target cells' adhesion is significant during the invasion process; after contact is made, some molecules involved in this process have been identified, including glucose and N-acetyl-d-galactosamine (Gal/GalNAc) lectin, a 260-kDa heterodimeric cell-surface protein consisting of a 170-kDa heavy chain (hgl) linked to a 35/31-kDa light chain (Igl) (Babuta et al., 2020). the light subunit is beloved to be attached to the membranes by the glycophosphoinositol stabilizers and the lectin-260 kDa is complexed with an intermediate subunit 150 kDa. The heavy chain consists of the carbohydrate identification domain (CRD), which is introduced on the cell surface. Overexpression of the mutant heavy chain subunit is permanently absent of the N-terminal i.e., In animal models, CRDs frequently confer a dominant-negative phenotype with reduced adhesion and pathogenicity. Furthermore, light subunits play a role in pathogenicity. The expression of the mutant from lgl (Part of the C-terminal) appears a negative phenotype (Babuta et al., 2020; Katz et al., 2002). The heterogeneous junction of mutant lgl is formed with hgl, but this junction is functionally inactive. Added to the Gal/GalNAc lectin, some other cell surface molecules involved in the adhesion action have been discovered (Daniela et al., 2014). In one study, the Ca2+ binding site for interaction with the ligand was identified (Chadee et al., 1988). Another study was alike determined the Ca2+ binding location, and a mutant was created that lost the ability to bind Ca2+. Although the mutant protein retained its carbohydrate-binding function, it lost its ability to agglutinate erythrocytes, suggesting that some properties of the Gal/GalNAc lectin are modulated by Ca2+ ions, similar to the Ca2+-binding protein calreticulin (CRT) on the surface of a cell protein (Yadav et al., 2016). *E.histolytica* attaches to complement component 1q (C1q), which is involved in phagocytosis of apoptotic immune cells but does not attack or kill normal cells such as Chinese Hamster Ovary (CHO) cells (Vaithilingam et al., 2012).

4.3.1. Relationship of Cytolysis Of Target Cells and Ca2+:

Amoebic cells express many different genes that exhibit proteolytic activity (Siqueira-Neto et al., 2018). The cysteine protein 5 (Ehcp5) is gaining attention because it is localized on the cell surface and because there is no active homolog in the non-pathogen *E.dispar* (D. Singh et al., 2004). Porin-like proteins from *E.histolytica* are also involved in cytolysis performed by amoeba cells. Interaction of the interaction of *E.histolytica* with target cells is the dramatic increase in Ca2+ levels at the end after contact. Preventing Ca2+ Chan-Nelson target cell death induced by Gal/GalNAc lectin due to purified protein itself is believed to increase Ca2+ levels in target cells (Babuta et al., 2020; Ravdin et al., 1985a), but the pathway to release Ca2+ from the target cell upon contact with *E.histolytica* is unclear. Multifold studies have displayed that Ca2+ signaling shares the ability of *E.histolytica* to kill the target cell. Avert the increase of Ca2+ between the parasitic cells avert the start of the cytolytic process (Makioka et al., 2001; Ravdin et al., 1982, 1985b). The direct involvement of Ca2+ in amoebae wildness was observed when the upstream factor of regulatory element 3 was found to regulate gene expression of the virulence-associated genes. UREBP binds the promoter element of Gal/GalNAc lectin hgl5. It contains 2 Ca2+ -binding EF-hand booster and regulates negative transcription in the presence of Ca2+, meaning it only binds the DNA promoter in the deficiency of Ca2+. (Gilchrlst et al., 2003). The Ca2+ binding flaw dialogue showed a dominant phenotype and the cells expressing the mutated protein were further virulent. There is a possibility that URE3BP plays a major role in virulence since a large proportion of amoeba genes contain the URE3 form which is recognized by URE3BP and thus controls the expression of a variety

of genes (Gilchrist et al., 2010). An unusual endemic number of URE3BP appears in the plasma membrane of trophozoites regardless of which one is in the nucleus (Moreno et al., 2010)

4.4. Acetylcholine (ACh) Virulence Factors of *E.histolytica*:

ACh is released from the gut nervous system in response to enteritis and regulates digestive functions, including the transfer of epithelial ions on its membrane (Wood, 2008). Trophozoites *E.histolytica* can bind ACh and thus modify parasite virulence by remodeling cell structure, parasite movement, phagocytosis, and increased expression and secretion of virulence factors, thus promoting amoebic cytotoxic (Medina-Rosales et al., 2021). The trophozoites' invasion of the gut is promoted by causing damage to the gut system and inflammation of the gut during infection, thence ACh may conceivably be involved in triggering amoebiasis. One study showed that ACh binds to trophozoites membranes, thereby increasing parasite proliferation and chemical transformation resulting in cell structure remodeling, regulation of the expression, and virulence factors such as Gal/GalNA lectin, L220, Amebapore C, and CP, which improved the Amoeba's ability to destroy and invade host tissues (Diamond et al., 1978). There is a study that showed that the activities increase the expression of actin and increase the polymerization of actin (F-actin) in response to the physiological levels of ACh. The dynamics of the cellular structure of the *E. histolytica* cytoskeleton are indispensable for cellular processes like adhesion, migration, phagocytosis, and killing of host cells. The host cells of *E.histolytica* are killed in a contact-dependent and contact-independent manner (Manich et al., 2018; Tavares et al., 2005). ACh upregulation of virulence factors, particularly secreted soluble components, resulted in a great increase in cellular toxicity of ACh-treated trophozoites against HepG2 cell monolayers, as evidenced by increased expression of amebapore and CP, as well as their will activities, through contact-independent processes. ACh stimuli also segmented cytopathic damage to the HpG2 monolayer (contact-dependent), a process closely related to frequent trogocytosis and phagocytosis (Talamás-Lara et al., 2014). Erythrocytosis is certainly the characteristic toxicity of pathogenic amoebas, which involves adhesion, cytoskeletal reorganization, and secretion of soluble agents that mediate host cell killing and ultimately ingest cellular debris. Ach supports erythrocytic activity in treated activities by increasing Gal/GalNAc and protein expression L220 (Manich et al., 2018; Medina-Rosales et al., 2021)

5. The Latest Drugs for Amebiasis:

E. histolytica may withstand current metronidazole (MTZ) treatment, and enhanced production of iron-containing superoxide dismutase and peroxiredoxin has been linked to drug resistance. MTZ resistance has been discovered in some clinical strains of *E.histolytica*, showing that the generation of drug-resistant strains is linked to enhanced production of iron-containing superoxide dismutase and peroxiredoxin. Molecular resistance to MTZ has been described in some clinical strains (Debnath et al., 2019). These observations led to the search for new drugs, and studies based on drug testing were published. The first study identified 1H-1,2,3-triazole-linked isatin-metronidazole conjugates that are most potent against *E.histolytica* and *Giardia lamblia* of MTZ (Kumar et al., 2018). The second study, E, targeting the primary biosynthetic pathway of the parasite cysteine, described the fungal metabolite as a compound that inhibits cysteine and amoeba growth in a cysteine-dependent manner with low mammalian cytotoxicity (Mori et al., 2018). A third study identified drugs capable of killing Riptaceae and MNZ resistant *E.histolytica* (Ehrenkaufer et al., 2018). In another study, farnesyltransferase (FT), the final co-enzyme of the mevalonate pathway-derived product function, was targeted. This enzyme is critical for a variety of functions including cell differentiation and growth (Probst et al., 2019). A promising therapy option for amebiasis is a synergistic

combination of metronidazole and the FT inhibitor lonafarnib. In many regions of the world, plants and their extracts are used to treat gastrointestinal problems. (Kelber et al., 2017).

Conclusions

E.histolytica virulence factors are essential for host-parasite interaction due to the severity of infection and tissue invasion. These factors can be modified by the parasite and the host to create a suitable environment for that parasite to live. Ca2+ is an important virulent factor. Calcium is known to be involved in many cellular processes in all eukaryotic systems. It regulates a large number of E.histolyitca signaling pathways that affect the virulence of the parasite. In addition to iron, E.histolyitca has an iron response. Two proteins (Ehhmb25 and Ehhmbp45) are involved in extracting iron from the blood, so it can be considered one of the virulences. The enteric nervous system releases ACh in response to intestinal inflammation, and ACh can modulate pathogenesis. EhGEF is involved in phagocytosis and plays an important role in endocytosis dependent on these proteins. EhGEF is a protein that binds to EhRho1 and interacts with it. Most virulence factors are up or downregulated by these factors, and their functions in *E. histolytica* are not yet clear. We encouragereaserchers to study their functions to understand more about the virulence of the parasite to develop a treatment for this disease.

Declarations:

Ethics approval and consent to participate: No application

Availability of data and materials: data available, Complete experimental details and data sets are available at the ‘‘ArrayExpress’’ MIAME-based database (www.ebi.ac.uk/arrayexpress/) with the accession number E-MTAB-1158.

Competing interests: The author declare that she has no competing interests

Funding: This paper was not supported by any funding

Authors' contribution: Author Ghaida Al-hazzaa, design of the work,Collected the data,Drafting the article and Final approval of the version to be published.

List of abbreviations

Abbreviation	Definition	Aberration	Definition
Fe	Iron	Hb	Hemoglobin
Ft	Ferritin	CPs	Carbamoyl Phosphate synthase II
GEF	Guanine nucleotide exchange factors	Rho	Ras homologous
Ca2+	Calcium	MTZ	Metrondizole
hgl	Heavy chain-linked	Lgl	Light subunite
CRD	Carbohydrate recognition	Ach	Acetylcholine

REFERENCES

Abubakar, A., Balla, H. J., Abdullahi, I. N., Zakari, M. M., Umar, K., Musa, M. H., & Yunusa, T. (2020). Intestinal Amebiasis and associated risk factors among pregnant Women attending University of Maiduguri Teaching Hospital, Nigeria. *Tanzania Medical Journal*, *31*(1), 30–47. https://doi.org/10.4314/TMJ.V31I1.331

Anaya-Velázquez, F., & Padilla-Vaca, F. (2011). Virulence of Entamoeba histolytica: a challenge for human health research. *Future Microbiology*, *6*(3), 255–258. https://doi.org/10.2217/FMB.11.2

Babuta, M., Bhattacharya, S., & Bhattacharya, A. (2020). Entamoeba histolytica and pathogenesis: A calcium connection. *PLoS Pathogens*, *16*(5). https://doi.org/10.1371/JOURNAL.PPAT.1008214

Betanzos, A., Bañuelos, C., & Orozco, E. (2019). Host Invasion by Pathogenic

Amoebae: Epithelial Disruption by Parasite Proteins. *Genes*, *10*(8). https://doi.org/10.3390/GENES10080618

Bharadwaj, R., Arya, R., Shahid mansuri, M., Bhattacharya, S., & Bhattacharya, A. (2017). EhRho1 regulates plasma membrane blebbing through PI3 kinase in Entamoeba histolytica. *Cellular Microbiology*, *19*(10), e12751. https://doi.org/10.1111/CMI.12751

Bharadwaj, R., Kushwaha, T., Ahmad, A., Inampudi, K. K., Nozaki, T., & Somlata. (2021). An atypical EhGEF regulates phagocytosis in Entamoeba histolytica through EhRho1. *PLoS Pathogens*, *17*(11), e1010030. https://doi.org/10.1371/JOURNAL.PPAT.1010030

Bharadwaj, R., Sharma, S., Arya, R., Bhattacharya, S., & Bhattacharya, A. (2018). EhRho1 regulates phagocytosis by modulating actin dynamics through EhFormin1 and EhProfilin1 in Entamoeba histolytica. *Cellular Microbiology*, *20*(9), e12851. https://doi.org/10.1111/CMI.12851

Bhattacharya, A., Padhan, N., Jain, R., & Bhattacharya, S. (2006). Calcium-Binding Proteins of Entamoeba histolytica. *Archives of Medical Research*, *37*(2), 221–225. https://doi.org/10.1016/J.ARCMED.2005.10.002

Bosch, D. E., Yang, B., & Siderovski, D. P. (2012). Entamoeba histolytica rho1 regulates actin polymerization through a divergent, diaphanous-related formin. *Biochemistry*, *51*(44), 8791–8801. https://doi.org/10.1021/BI300954G/SUPPL_FILE/BI300954G_SI_008.PDF

Buchsbaum, R. J. (2007). Rho activation at a glance. *Journal of Cell Science*, *120*(7), 1149–1152. https://doi.org/10.1242/JCS.03428

Burton Bogotá's., Clint Carter., & Thomas Oeltmann. (2012). Human Parasitology 4th Edition

Carol J.Baker., MD., & FAAP. (2017). Red book Atlas Of Pediatrics Infectious Disease, 3rd Edition

Chadee, K., Johnson, M. L., Orozco, E., Petri, W. A., & Ravdin, J. I. (1988). Binding and internalization of rat colonic mucins by the galactose/N-acetyl-D-galactosamine adherence lectin of Entamoeba histolytica. *The Journal of Infectious Diseases*, *158*(2), 398–406. https://doi.org/10.1093/INFDIS/158.2.398

Chakraborty, P. (2004). *Textbook of medical parasitology*. 358. https://www.goodreads.com/work/best_book/54548254-textbook-of- medical-parasitology

Cruz-Castañeda, A., Hernández-Sánchez, J., & Olivares-Trejo, J. J. (2009). Cloning and identification of a gene coding for a 26-kDa hemoglobin-binding protein from Entamoeba histolytica. *Biochimie*, *91*(3), 383–389. https://doi.org/10.1016/J.BIOCHI.2008.10.016

Cruz-Castañeda, A., López-Casamichana, M., & Olivares-Trejo, J. J. (2011). Entamoeba histolytica secretes two haem-binding proteins to scavenge haem. *Biochemical Journal*, *434*(1), 105–111. https://doi.org/10.1042/BJ20100897

Cui, Z., Li, J., Chen, Y., & Zhang, L. (2019). Molecular epidemiology, evolution, and phylogeny of Entamoeba spp. *Infection, Genetics and Evolution*, *75*, 104018. https://doi.org/10.1016/J.MEEGID.2019.104018

Debnath, A., Rodriguez, M. A., & Ankri, S. (2019a). Editorial: Recent Progresses in Amebiasis. *Frontiers in Cellular and Infection Microbiology*, *9*(JUL). https://doi.org/10.3389/FCIMB.2019.00247

Debnath, A., Rodriguez, M. A., & Ankri, S. (2019b). Editorial: Recent progresses in amebiasis. *Frontiers in*

Cellular and Infection Microbiology, *9*(JUL), 247. https://doi.org/10.3389/FCIMB.2019.00247/BIBTEX

Despommier, D. D., Griffin, D. O., Gwadz, R. W., Hotez, P. J., Knirsch, C. A., Photographs, J. K., & Griffin, D. (2017). *Parasitic Diseases Sixth Edition*. www.parasiteswithout borders .com

Diamond, L. S., Harlow, D. R., & Cunnick, C. C. (1978). A new medium for the axenic cultivation of Entamoeba histolytica and other Entamoeba. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *72*(4), 431–432. https://doi.org/10.1016/0035-9203(78)90144-X

Domínguez-Fernández, T., Rodríguez, M. A., Sánchez Monroy, V., Gómez García, C., Medel, O., & Pérez Ishiwara, D. G. (2018). A Calpain-Like Protein Is Involved in the Execution Phase of Programmed Cell Death of Entamoeba histolytica. *Frontiers in Cellular and Infection Microbiology*, *8*, 339. https://doi.org/10.3389/FCIMB.2018.00339/BIBTEX

Dong, N., Liu, L., & Shao, F. (2010). A bacterial effector targets host DH-PH domain RhoGEFs and antagonizes macrophage phagocytosis. *The EMBO Journal*, *29*(8), 1363–1376. https://doi.org/10.1038/EMBOJ.2010.33

Ehrenkaufer, G. M., Suresh, S., Solow-Cordero, D., & Singh, U. (2018). High-throughput screening of Entamoeba identifies compounds which target both life cycle stages and which are effective against metronidazole resistant parasites. *Frontiers in Cellular and Infection Microbiology*, *8*(AUG), 276. https://doi.org/10.3389/FCIMB.2018.00276/BIBTEX

García-Mata, R., & Burridge, K. (2007). Catching a GEF by its tail. *Trends in Cell Biology*, *17*(1), 36–43. https://doi.org/10.1016/J.TCB.2006.11.004

Gastelum-Martínez, A., León-Sicairos, C., Plata-Guzmán, L., Soto-Castro, L., León-Sicairos, N., & de La Garza, M. (2018). Iron-modulated virulence factors of Entamoeba histolytica. *Future Microbiology*, *13*(11), 1329–1341. https://doi.org/10.2217/FMB-2018-0066

Gilchrist, C. A., Moore, E. S., Zhang, Y., Bousquet, C. B., Lannigan, J. A., Mann, B. J., & Petri, W. A. (2010). Regulation of virulence of Entamoeba histolytica by the URE3-BP transcription factor. *MBio*, *1*(1). https://doi.org/10.1128/MBIO.00057-10/ASSET/E178CFA7-33FF-43AF-B8CA-E628B6FA567F/ASSETS/GRAPHIC/MBO9991010050005.JPEG

Gilchrlst, C. A., Leo, M., Line, C. G., Mann, B. J., & Petri, W. A. (2003). Calcium Modulates Promoter Occupancy by the Entamoeba histolytica Ca2+-binding Transcription Factor URE3-BP *. *Journal of Biological Chemistry*, *278*(7), 4646–4653. https://doi.org/10.1074/JBC.M211271200

Guerrant, R. L., Walker, D. H., & Weller, P. F. (2011). Tropical Infectious Diseases: Principles, Pathogens and Practice, 3rd Edition: *Http://Dx.Doi.Org/10.1345/Aph.1Q302*, *45*(11), 1459–1460. https://doi.org/10.1345/APH.1Q302

Hall, A. B., Gakidis, M. A. M., Glogauer, M., Wilsbacher, J. L., Gao, S., Swat, W., & Brugge, J. S. (2006). Requirements for Vav guanine nucleotide exchange factors and Rho GTPases in FcγR- and complement-mediated phagocytosis. *Immunity*, *24*(3), 305–316. https://doi.org/10.1016/J.IMMUNI.2006.02.005/ATTACHMENT/4E079D51-E78F-4367-9E94-BBF49E0EB50E/MMC1.PDF

Hernández-Cuevas, N. A., Weber, C., Hon, C. C., & Guillen, N. (2014). Gene Expression Profiling in Entamoeba

histolytica Identifies Key Components in Iron Uptake and Metabolism. *PLOS ONE*, *9*(9), e107102. https://doi.org/10.1371/JOURNAL.PONE.0107102

Hernandez-Flores, A., Almaraz-Barrera, M. de J., Lozano-Amado, D., Correa-Basurto, J., Rojo-Dominguez, A., Luna-Rivera, E., Schnoor, M., Guillen, N., Hernandez-Rivas, R., & Vargas, M. (2016). A new nucleocytoplasmic RhoGAP protein contributes to control the pathogenicity of Entamoeba histolytica by regulating EhRacC and EhRacD activity. *Cellular Microbiology*, *18*(11), 1653–1672. https://doi.org/10.1111/CMI.12603https://doi.org/10.1093/JB/MVW024

Hunter's Tropical Medicine and Emerging Infectious Diseases. (2020). *Hunter's Tropical Medicine and Emerging Infectious Diseases*. https://doi.org/10.1016/C2016-001879-X

Kantor, M., Abrantes, A., Estevez, A., Schiller, A., Torrent, J., Gascon, J., Hernandez, R., & Ochner, C. (2018). Entamoeba Histolytica: Updates in Clinical Manifestation, Pathogenesis, and Vaccine Development. *Canadian Journal of Gastroenterology & Hepatology*, *2018*. https://doi.org/10.1155/2018/4601420

Katz, U., Ankri, S., Stolarsky, T., Nuchamowitz, Y., & Mirelman, D. (2002). Entamoeba histolytica expressing a dominant negative N-truncated light subunit of its Gal-lectin are less virulent. *Molecular Biology of the Cell*, *13*(12), 4256–4265. https://doi.org/10.1091/MBC.E02-06-0344/ASSET/IMAGES/LARGE /MK1222035008.JPEG

Kelber, O., Bauer, R., & Kubelka, W. (2017). Phytotherapy in Functional Gastrointestinal Disorders. *Digestive Diseases*, *35*(S1), 36–42. https://doi.org/10.1159/000485489

Kumar, S., Bains, T., Won Kim, A. S., Tam, C., Kim, J., Cheng, L. W., Land, K. M., Debnath, A., & Kumar, V. (2018). Highly Potent 1H-1,2,3-Triazole-Tethered Isatin-Metronidazole Conjugates Against Anaerobic Foodborne, Waterborne, and Sexually-Transmitted Protozoal Parasites. *Frontiers in Cellular and Infection Microbiology*, *8*, 380. https://doi.org/10.3389/FCIMB.2018.00380/BIBTEX

Lee, J., Park, S. J., & Yong, T. S. (2008). Effect of iron on adherence and cytotoxicity of Entamoeba histolytica to CHO cell monolayers. *The Korean Journal of Parasitology*, *46*(1), 37–40. https://doi.org/10.3347/KJP.2008.46.1.37

Lee, W. L., Cosio, G., Ireton, K., & Grinstein, S. (2007). Role of CrkII in Fcγ Receptor-mediated Phagocytosis *. *Journal of Biological Chemistry*, *282*(15), 11135–11143. https://doi.org/10.1074/JBC.M700823200

Makioka, A., Kumagai, M., Ohtomo, H., Kobayashi, S., & Takeuchi, T. (2001). Effect of calcium antagonists, calcium channel blockers and calmodulin inhibitors on the growth and encystation of Entamoeba histolytica and E. invadens. *Parasitology Research 2001 87:10*, *87*(10), 833–837. https://doi.org/10.1007/S004360100453

Manich, M., Hernandez-Cuevas, N., Ospina-Villa, J. D., Syan, S., Marchat, L. A., Olivo-Marin, J. C., & Guillén, N. (2018). Morphodynamics of the actin-rich cytoskeleton in Entamoeba histolytica. *Frontiers in Cellular and Infection Microbiology*, *8*(MAY), 179. https://doi.org /10.3389/FCIMB.2018.00179/BIBTEX

Mao, Y., & Finnemann, S. C. (2015). Regulation of phagocytosis by Rho GTPases. Https://Doi.Org/10.4161/21541248.2014.989785, *6*(2), 89–99. https://doi.org/10.4161/21541248.2014.989785

````
````

Medina-Rosales, M. N., Muñoz-Ortega, M. H., García-Hernández, M. H., Talamás-Rohana, P., Medina-Ramírez, I. E., Salas-Morón, L. G., Martínez-Hernández, S. L., Ávila-Blanco, M. E., Medina-Rosales, B., & Ventura-Juárez, J. (2021). Acetylcholine Upregulates Entamoeba histolytica Virulence Factors, Enhancing Parasite Pathogenicity in Experimental Liver Amebiasis. *Frontiers in Cellular and Infection Microbiology*, *10*, 1. https://doi.org/10.3389/FCIMB.2020.586354/FULL

Monroy, V. S., Flores, O. M., García, C. G., Maya, Y. C., Fernández, T. D., & Pérez Ishiwara, D. G. (2015). Calpain-like: A Ca2+ dependent cystein protease in Entamoeba histolytica cell death. *Experimental Parasitology*, *159*, 245–251. https://doi.org/10.1016/J.EXPPARA.2015.10.005

Moreno, H., Linford, A. S., Gilchrist, C. A., & Petri, W. A. (2010). Phospholipid-binding protein EhC2A mediates calcium-dependent translocation of transcription factor URE3-BP to the plasma membrane of Entamoeba histolytica. *Eukaryotic Cell*, *9*(5), 695–704. https://doi.org/10.1128/EC.00346-09/SUPPL_ FILE/ HMORENO _SUP_EC.PDF

Mori, M., Tsuge, S., Fukasawa, W., Jeelani, G., Nakada-Tsukui, K., Nonaka, K., Matsumoto, A., Ōmura, S., Nozaki, T., & Shiomi, K. (2018). Discovery of Antiamebic Compounds That Inhibit Cysteine Synthase From the Enteric Parasitic Protist Entamoeba histolytica by Screening of Microbial Secondary Metabolites. *Frontiers in Cellular and Infection Microbiology*, *8*, 409. https://doi.org/10.3389/FCIMB.2018.00409/BIBTEX

Nozaki, T., & Bhattacharya, A. (2015). Amebiasis: Biology and pathogenesis of entamoeba. *Amebiasis: Biology and Pathogenesis of Entamoeba*, 1–581. https://doi.org/10.1007/978-4-431-55200-0

Paniker's. (2018). Textbook of Medical Parasitology 8th Edition

Pritt, B. S., & Graham Clark, C. (2008). Amebiasis. *Mayo Clinic Proceedings*, *83*(10), 1154–1160. https://doi.org/10.4065/83.10.1154

Probst, A., Nguyen, T. N., El-Sakkary, N., Skinner, D., Suzuki, B. M., Buckner, F. S., Gelb, M. H., Caffrey, C. R., & Debnath, A. (2019). Bioactivity of farnesyltransferase inhibitors against entamoeba histolytica and schistosoma mansoni. *Frontiers in Cellular and Infection Microbiology*, *9*(MAY), 180. https://doi.org/ 10.3389/FCIMB.2019.00180/BIBTEX

Ravdin, J. I., Murphy, C. F., Guerrant, R. L., & Long-Krug, S. A. (1985a). Effect of Antagonists of Calcium and Phospholipase A on the Cytopathogenicity of Entamoeba histolytica. *The Journal of Infectious Diseases*, *152*(3), 542–549. https://doi.org/10.1093/INFDIS/152.3.542

Ravdin, J. I., Murphy, C. F., Guerrant, R. L., & Long-Krug, S. A. (1985b). Effect of Antagonists of Calcium and Phospholipase A on the Cytopathogenicity of Entamoeba histolytica. *The Journal of Infectious Diseases*, *152*(3), 542–549. https://doi.org/10.1093/INFDIS/152.3.542

Ravdin, J. I., Sperelakis, N., & Guerrant, R. L. (1982). Effect of Ion Channel Inhibitors on the Cytopathogenicity of Entamoeba histolytica. *The Journal of Infectious Diseases*, *146*(3), 335–340. https://doi.org/10.1093/INFDIS/146.3.335

Rogier, E. W., Frantz, A. L., Bruno, M. E. C., & Kaetzel, C. S. (2014). Secretory IgA is Concentrated in the Outer Layer of Colonic Mucus along with Gut Bacteria. *Pathogens (Basel, Switzerland)*, *3*(2), 390–403. https://

doi.org/10.3390/PATHOGENS3020390

Samie, A., ElBakri, A., & AbuOdeh, R. (2012). Amoebiasis in the Tropics: Epidemiology and Pathogenesis. *Current Topics in Tropical Medicine*. https://doi.org/10. 5772/ 26810

Singh, A., Houpt, E., & Petri, W. A. (2009). Rapid Diagnosis of Intestinal Parasitic Protozoa, with a Focus on Entamoeba histolytica. *Interdisciplinary Perspectives on Infectious Diseases*, *2009*, 1–8. https ://doi.org/10.1155/2009/547090

Singh, D., Naik, S. R., & Naik, S. (2004). Role of cysteine proteinase of Entamoeba histolytica in target cell death. *Parasitology*, *129*(2), 127–135. https://doi.org/10.1017/ S0031182004005451

Siqueira-Neto, J. L., Debnath, A., McCall, L. I., Bernatchez, J. A., Ndao, M., Reed, S. L., & Rosenthal, P. J. (2018). Cysteine proteases in protozoan parasites. *PLoS Neglected Tropical Diseases*, *12*(8). https:// doi.org/10.1371/JOURNAL.PNTD. 0006512

Song, S., Li, X., Geng, C., Li, Y., & Wang, C. (2020). Somatostatin stimulates colonic MUC2 expression through SSTR5-Notch-Hes1 signaling pathway. *Biochemical and Biophysical Research Communications*, *521*(4), 1070–1076. https://doi.org/10.1016/J. BBRC.2019.11.034

Talamás-Lara, D., Chávez-Munguía, B., González-Robles, A., Talamás-Rohana, P., Salazar-Villatoro, L., Durán-Díaz, Á., & Martínez-Palomo, A. (2014). Erythrophagocytosis in Entamoeba histolytica and Entamoeba dispar: A comparative study. *BioMed Research International*, *2014*. https: //doi.org/10.1155/2014/626259

Tavares, P., Rigothier, M. C., Khun, H., Roux, P., Huerre, M., & Guillén, N. (2005). Roles of Cell Adhesion and Cytoskeleton Activity in Entamoeba histolytica Pathogenesis: a Delicate Balance. *Infection and Immunity*, *73*(3), 1771. https://doi.org/10. 1128/IAI.73.3.1771-1778.2005

Toma-Fukai, S., & Shimizu, T. (2019). Structural Insights into the Regulation Mechanism of Small GTPases by GEFs. *Molecules 2019, Vol. 24, Page 3308*, *24*(18), 3308. https://doi.org/10.3390/MOLECULES24183308

Vaithilingam, A., Teixeira, J. E., Miller, P. J., Heron, B. T., & Huston, C. D. (2012). Entamoeba histolytica cell surface calreticulin binds human C1q and functions in amebic phagocytosis of host cells. *Infection and Immunity*, *80*(6), 2008–2018. https://doi.org/10.1128/IAI.06287-11/ ASSET/394ACACE-8F2C-4FBD-BBCF-D11D1598C09C/ ASSETS/GRAPHIC/ZII999099685000 6.JPEG

Wood, J. D. (2008). Enteric nervous system: Reflexes, pattern generators and motility. *Current Opinion in Gastroenterology*, *24*(2), 149–158. https://doi.org/10.1097/MOG.0B013E3282F56125

Xu, J. D., Diao, M. Q., Niu, G. J., Wang, X. W., Zhao, X. F., & Wang, J. X. (2018). A Small GTPase, RhoA, Inhibits Bacterial Infection Through Integrin Mediated Phagocytosis in Invertebrates. *Frontiers in Immunology*, *9*, 1928. https://doi. org/10.3389/FIMMU.2018.01928/BIBTEX

Yadav, R., Verma, K., Chandra, M., Mukherjee, M., & Datta, S. (2016). Biophysical studies on calcium and carbohydrate binding to carbohydrate recognition domain of Gal/GalNAc lectin from Entamoeba histolytica: insights into host cell adhesion. *Journal of Biochemistry*, *160*(3), 177–186.

````
````