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Research Article

Assessment of Virulence Gene Expression in Entamoeba Strains from Basrah, Southern Iraq

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About Article

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ABSTRACT

Entamoeba histolytica is the primary protozoan parasite causing amoebiasis, a disease characterized by diarrhea and dysentery. *Entamoeba histolytica* trophozoites develop a virulent phenotype that leads to intestinal tissue invasion and the onset of amoebiasis symptoms. Quantitative real-time PCR (qRT-PCR) was performed to quantify the expression levels of selected virulence genes cysteine protease (CP), amoebapore B (AP), and Gal/GalNAc lectin (GAL) in *Entamoeba* strains. Our findings showed that assessing the expression of three key virulence genes cysteine protease (CP), amoebapore (AP), and Gal/GalNAc lectin (GAL) in *Entamoeba* strains. The results revealed low CP expression across all strains, suggesting reduced invasive ability. High AP expression was observed in DM24, DM33 for *E. moshkovskii* strains, indicating strong cytolytic potential, while *E. histolytica* DM5 showed lower AP levels. GAL expression was elevated in *E. histolytica* DM1 and DM5 and *E. moshkovskii* DM33, pointing to effective adhesion, but was significantly lower in *E. moshkovskii* DM24, indicating impaired virulence. Overall, the study highlights strain-specific differences in virulence gene expression among *Entamoeba* strains. The study focused on evaluating the expression of key virulence genes in *Entamoeba* strains from Basrah, highlighting strain-specific differences that influence their pathogenicity. By analyzing genes involved in tissue invasion, cytolysis, and adhesion, the research provides insights into the varying disease-causing potential of different

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1. INTRODUCTION

Amoebiasis is caused by *Entamoeba histolytica*, a protozoan parasite transmitted via ingestion of cysts in contaminated food or water (Lohia, 2003). The infection is more common in children as well as adults, and it is widely spread in the subtropical and tropical areas (Ohnishi *et al.*, 2004). It has been shown that about 450 million persons were quite an infection annually, with about of 50 million deaths (Ravdin & Petri, 1995). The host–parasite interaction in amoebiasis exhibits considerable variability, ranging from a commensal relationship, as observed in asymptomatic cyst carriers, to pathogenic interactions in which the parasite induces varying degrees of tissue damage. In severe cases, this damage may progress to the invasion of extra-intestinal organs (WHO, 1997; Mortimer & Chadee, 2010; Haque *et al.*, 2003).

This study aims to investigate the virulence factors of different genotypes of *Entamoeba* species by analyzing the gene expression profiles of three key virulence genes: cysteine protease (CP), amoebapore (AP), and Gal/GalNAc lectin (GAL). This approach aims to provide insights into the pathogenic potential of these genotypes.

2. LITERATURE REVIEW

The pathogenesis of parasitic diseases is highly variable, influenced by factors such as parasite size, nutritional requirements, individual life cycles, and immunologic consequences of infection, as noted by Ryan (2017). Trophozoites can colonize the bowel lumen as commensal flora or invade the colonic epithelium, causing inflammation and bowel wall destruction. Factors influencing invasion include genotype variation, host genetic differences, gut microbiome differences, nutritional status, and immunocompetence. The determinants of invasion remain unknown (Royer & Petri, 2014) .*E. histolytica* parasites produce enzymes such as lipases and cysteine proteases that degrade epithelial cells, leading to cellular necrosis and apoptosis. These enzymes, together with other virulence factors including collagenase and additional cysteine proteases, contribute significantly to tissue invasion and pathogenesis (Dolabella *et al.*, 2012).

The adhesion of *Entamoeba* to epithelial cells, the basement membrane, and colonic mucin glycoproteins is mediated primarily by the Gal/GalNAc lectin, which binds specifically to host galactose and N-acetyl-D-galactosamine residues. This initial attachment is a critical step in colonization and pathogenesis. In addition, virulence factors such as amoebapores (AP) play a key role in cytotoxicity by forming pores in host cell membranes, leading to ion imbalance and extracellular Ca^{2+} influx, which contribute to cell lysis and death. Together, Gal/GalNAc lectin and amoebapores enhance the parasite's ability to adhere, invade, and resist host immune responses, including complement-mediated lysis (Saha *et al.*, 2015; Burgess *et al.*, 2017).

3. METHODOLOGY

3.1. Experimental infection

3.1.1. Cultivations of *Entamoeba* spp.

Entamoeba histolytica and *E. moshkovskii* were isolated from stool samples and cultivated in TYI-S-33 medium. Four strains were selected for analysis, including DM1 and DM5 (representing different genotypes of *E. histolytica*) and DM24 and DM33 (representing different genotypes of *E. moshkovskii*) after two days of cultivation.

3.1.2. Inoculation of rats and assessment of results

Five male albino Wistar rats (8–10 weeks old; 50–70 g) were obtained from the College of Veterinary Medicine, University of Basrah. Animals were screened to exclude intestinal parasitic infections and housed under standard laboratory conditions with free access to food and water. The animals were divided into two groups; the infected group was orally inoculated with *Entamoeba* spp. cultured in TYI-S-33 medium, containing 600 trophozoites/mL (Diamond *et al.*, 1978). After 21 days, animals were anesthetized with a ketamine/xylazine cocktail (87.5 mg/kg and 12.5 mg/kg, respectively) (Essa Aledani *et al.*, 2020) and euthanized following ethical guidelines. Cecal tissues were collected postmortem for histopathological analysis, processed using the method of by Bancroft and Gamble (2008).

3.2. Histopathological examination Light microscope study

Histological processing of tissue samples was performed based on standard procedures described by Bancroft and Gamble (2008), with minor modifications tailored to the experimental setup to detect the presence and note the effect of *Entamoeba* spp.

3.3. Total RNA extraction

Total RNAs were extracted from Rat cecal tissues were harvested and immediately preserved in RNAlater solution to stabilize RNA for subsequent molecular Total RNA was extracted using the Easy-spin™ (DNA free) Total RNA Extraction Kit (South Korea) according to the manufacturer's instruction.

3.4. Revers transcription of RNA

cDNA was synthesized from total RNA using the AddScript cDNA Synthesis Kit (Addbio, South Korea) following the manufacturer's protocol. Each reaction contained 164 ng of RNA (4 µL) in a final volume of 20 µLThe reaction tubes were placed in a thermal cycler and subjected to the following program: priming at 25°C for 10 minutes, reverse transcription at 50°C for 60 minutes, enzyme inactivation at 80°C for 5 minutes, and finally held at 12°C indefinitely

3.5. Real-Time Quantitative PCR (RT-qPCR)

Quantitative real-time PCR (qRT-PCR) was performed to quantify the expression levels of selected virulence genes cysteine protease (CP), amoebapore B (AP), and Gal/GalNAc lectin (GAL) in *Entamoeba* strains. Actin-alpha (ACT) was used as a reference gene for data normalization in gene expression analysis. cDNA was used as the template for qRT-PCR with SYBR Green PCR reagents.

Each qRT-PCR reaction was carried out in a total volume of 20 µL, which included 2 µL of cDNA template from each sample, gene-specific primers, Preparation of Real-Time PCR The total reaction volume for the qPCR assay was adjusted to 20 µl. Each reaction mixture contained 10 µl of GoTaq™ qPCR Master Mix (2X), 2 µl of the forward primer, 2 µl of

the reverse primer, 4 µl of nuclease-free water (ddH_2O), and 2 µl of cDNA template.The primers used in this study for the amplification of target genes included those specific for cysteine protease (CP), amoebapore B (AP), and Gal/GalNAc lectin (GAL), with actin-alpha serving as the housekeeping gene for normalization. The sequences of the primers were as follows: for cysteine protease (CP), the forward primer (CP-F) was 5′-GCTGTTGCTGGTACTTGCAAG-3′ and the reverse primer (CP-R) was 5′-ACAGCAACAGGTCCGTTTTC-3′ (Accession No. M27307.1; Al-Abodi *et al.*, 2015). For amoebapore B (AP), the forward primer (AP-F) was 5′-TGCCTTTGCTGCAACAAGAG-3′ and the reverse primer (AP-R) was 5′-ACAGCTTGAGCACCATCAAC-3′ (Accession No. X76904.1; Al-Abodi *et al.*, 2015). For Gal/GalNAc lectin (GAL), the forward primer (GL-F) was 5′-GACGCACCAGGTACTCAAAATC-3′ and the reverse primer (GL-R) was 5′-AACCCATCTTCCACCCTGATTG-3′ (Accession No. XM_001736121.1; Al-Abodi *et al.*, 2015). Finally, for the actin-alpha gene, the forward primer (Act-F) was 5′-AGCTGTTCTTTCATTATATGC-3′ and the reverse primer (Act-R) was 5′-TTCTCTTTCAGCAGTAGTGGT-3′ (Accession No. AP023114.1; González-Rivas *et al.*, 2020)and the appropriate master mix components. Amplification was performed on a real-time PCR system under the thermal cycling conditions described in Table.

Table 1. qRT-PCR thermal cycling conditions

NO.	Stage	Ta (°C)	Time	Cycles
1.	Hot-start activation	95	5 min	1X
2.	Denaturation	95	10 sec.	40X
3.	Annealing	60	30 sec.	
4.	Extension	72	30 sec.	
5.	Dissociation	72	2 min	1X

$\Delta CT_{infection} = CT_{target\ gene} - CT_{HK\ gene}$
$\Delta CT_{control} = CT_{target\ gene} - CT_{HK\ gene}$
$\Delta\Delta CT = \Delta CT_{infection} - \Delta CT_{control}$
Gene expression (E) = $2^{-\Delta\Delta CT}$
Fold change = (Exp. of infection/Exp. of control)

3.6. Statistical analysis

"Statistical analysis was carried out using GraphPad Prism version 10 (GraphPad Software Inc.). A one-way ANOVA test was employed to assess the differences in expression levels of the three virulence genes—cysteine protease (CP), amoebapore (AP), and Gal/GalNAc lectin (GL). The comparison was made among different *Entamoeba* strains representing the genotypes *E. histolytica* and *E. moshkovskii.*

4. RESULTS AND DISCUSSION

The present study demonstrated that the cecal tissue showed a heavy infection with *E. histolytica*, with numerous trophozoites indicating active parasitic invasion. Extensive sloughing of the epithelial lining, disruption of normal mucosal architecture, and destruction in multiple areas were observed, indicating the invasive nature of *E. histolytica* infection (Figure 1 & Figure 2) Histological examination of cecum sections from rats infected with E.moshkovskii showed trophozoites on the mucosal layer, mild epithelial cell damage, and normal intestinal gland architecture Figure (3). In contrast, cecum sections from a control group showed a normal tissue structure Figure (4)

The histological findings demonstrate a clear difference in pathogenic behavior between *E. histolytica* and *E. moshkovskii.* Tissues infected with *E. histolytica* showed significant damage and deep invasion, likely due to its production of proteolytic enzymes and strong adhesion mediated by the Gal/GalNAc lectin, reflecting its virulent nature. In contrast, *E. moshkovskii* was found only on the epithelial surface without signs of invasion or notable tissue damage, indicating a non-invasive and less pathogenic profile. These results highlight the importance of histological analysis in distinguishing invasive from non-invasive *Entamoeba* species. Our study is consistent with Al-Hilfi (2020), showing that *E. histolytica* invades tissue layers, causing histopathological damage, whereas *E. moshkovskii* remains on the epithelial surface without deep tissue injury, highlighting pathological differences and the importance of histopathology in distinguishing invasive from non-invasive *Entamoeba* species. The current study demonstrated differences in the expression of virulence genes—Cysteine protease (CP), Amoebopore (AP), and galactose/N-acetyl-D-galactosamine lectin (GL)—between strains DM1 and DM5, belonging to genotypes 1 and 2, respectively, isolated from different regions of Basrah (Al-Qibla and Abu Al-Khasib Center) for *E. histolytica.* Similarly, strains DM24 and DM33, belonging to genotypes 1 and 2, respectively, were isolated from different regions of Basrah (Abu Al-Khasib - Abahadria and Abu Al-Khasib Centre) for *E. moshkovskii.*

All *Entamoeba* spp. strains were evaluated in comparison with the healthy control group to determine variations in CP, AP, and GL gene expression. One peak melting curves for genes were obtained to illustrate the specific binding of primers with the target gene, Figure (6) and melt cruve Figure (5).

For the CP gene, the highest expression was observed in the control group (1.000 ± 0.000), while all *Entamoeba* strains showed significant downregulation ($P < 0.05$). CP gene expression was significantly downregulated in all *Entamoeba* strains compared to the control (1.000 ± 0.000), with *E. histolytica* DM1 at 0.2082 ± 0.0102, DM5 at 0.2111 ± 0.0103, and *E. moshkovskii* DM24 and DM33 at 0.3112 ± 0.0152 and 0.0382 ± 0.0034, respectively, indicating reduced CP-related activity across strains. For the AP gene, expression was highest in E. mashkovskii DM33 (39.75 ± 3.50), followed by DM24 (36.25 ± 0.36), *E. histolytica* DM1 (27.16 ± 2.66), and *E. histolytica* DM5 (5.70 ± 0.28). The control group recorded the lowest expression (1.00 ± 0.00). All differences were statistically significant ($P < 0.05$).GL gene expression was highest in *E. histolytica* DM5 (3.08 ± 0.30) and E. mashkovskii DM33 (3.08 ± 0.18), closely followed by *E. histolytica* DM1 (3.01 ± 0.06). E. mashkovskii DM24 showed markedly lower expression (0.36 ± 0.03), while the control group remained at baseline (1.00 ± 0.00). These variations were statistically significant ($P < 0.05$). Overall, the data demonstrate significant inter-strain differences, with uniform downregulation of CP and strain-specific upregulation of AP and GL Figure (7).

The observed variation in virulence gene expression among

Entamoeba strains underscores the complexity of pathogenic mechanisms within this genus. The consistently low expression of the CP gene suggests a general reduction in tissue-degrading capability, which may influence invasion efficiency. In contrast, the elevated AP expression in certain *E. moshkovskii* strains points to a heightened cytolytic activity that could compensate for lower protease levels. High GAL expression in some strains indicates strong adhesion potential, a critical factor for colonization and infection persistence. These differential expression patterns highlight the need for strain-specific approaches in diagnosing and managing amoebic infections.

Amebiasis is recognized as a significant global health concern, with *Entamoeba histolytica* identified as a major contributor to morbidity and mortality. Throughout its life cycle, *E. histolytica* is exposed to multiple environmental pressures, including oxidative and nitrosative stresses from the host immune response, shifts in oxygen and glucose availability, and alterations in gut microbiota (Vicente *et al.*, 2009; Nagaraja & Ankri, 2018). These factors demand robust adaptive mechanisms to ensure its persistence and pathogenicity within the host (Petri *et al.*, 2002). Cysteine proteinases (CPs) have been identified as critical virulence factors in *E. histolytica*, playing a key role in intestinal invasion through the degradation of the extracellular matrix and evasion of the host immune response. This study evaluated key virulence genes in four *Entamoeba* strains (*E. histolytica* DM1, DM5; *E. moshkovskii* DM24, DM33). CP was downregulated in all strains, suggesting reduced invasive capacity. AP showed high expression in *E. moshkovskii* DM24 and DM33, indicating strong cytolytic potential, while *E. histolytica* DM5 had lower levels. GAL expression was high in *E. histolytica* DM1, DM5, and *E. moshkovskii* DM33, but low in DM24, implying impaired adhesion. These results highlight strain-specific differences in virulence factor expression and pathogenic potential These enzymes are encoded by multiple genes, which are notably absent or minimally expressed in non-invasive strains. The lack of CP gene activation or low expression levels observed in studied groups is indicative of a non-invasive phenotype, given the strong association of cysteine proteases with tissue invasion and extraintestinal amoebiasis (Que & Reed, 2000).

The secretion of cysteine proteases has also been demonstrated to be an essential factor in *Entamoeba* pathogenicity. These enzymes are responsible for digesting extracellular matrix proteins, thereby facilitating deeper penetration of trophozoites into intestinal tissues and disruption of the mucosal barrier (Que & Reed, 2000). Furthermore, amoebapores, a family of small proteins produced by *E. histolytica*, have been characterized as functionally similar to granulolysin proteins found in natural killer cells and cytotoxic lymphocytes (Leippe, 1997; Andra *et al.*, 2003).

The Gal/GalNAc-lectin (Ehlect) has been implicated in the initial adherence of trophozoites to the intestinal mucosa and epithelial cells. It has been shown that these early interactions can be inhibited by monoclonal antibodies targeting the carbohydrate recognition domain within the Hgl subunit (Frederick & Petri, 2005; Bracha & Mirelman, 1983).

The variability in clinical outcomes of *Entamoeba histolytica* infections, ranging from asymptomatic colonization to tissue invasion, is considered to reflect the complexity of host-parasite interactions. A significant obstacle in elucidating the mechanisms of virulence, immune evasion, and environmental adaptation has been the lack of an experimental model that fully replicates.

Bansal *et al.* (2009) reported that even low levels of Gal/GalNAc lectin and amebapore gene expression did not inhibit *E. histolytica* invasion in a human ex vivo intestinal model, suggesting the presence of potential compensatory mechanisms. Similarly, Ximénez *et al.* (2017) observed elevated expression of virulence-associated genes—including EhGal/GalNAc lectin, Ehap-a, and several cysteine proteases—in liver tissues infected with *E. histolytica*, whereas tissues infected with E. dispar displayed reduced or absent expression of these genes. They also reported increased expression of survival-related genes such as peroxiredoxin, superoxide dismutase, and the heat shock protein Ehhsp-70, which protect the parasite from oxidative stress and host immune responses.

Furthermore, González-Rivas *et al.* (2020) demonstrated that overexpression of cysteine protease genes (Ehcp5 and Ehcp2) occurs in both amoebic liver abscesses and genital cutaneous amoebiasis, despite the differences in infection sites and environmental pressures. Collectively, these findings indicate that *E. histolytica* trophozoites modulate their gene expression in response to environmental stimuli including immune pressure and oxygen availability thereby adapting their pathogenicity across diverse clinical contexts

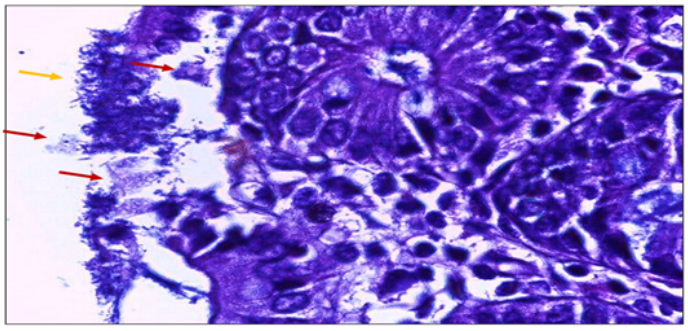

Figure 1. Histological section of the cecum from a rat infected with *Entamoeba histolytica* at 21 days post-infection. The image shows a heavy parasitic invasion (red arrow) and extensive epithelial desquamation (yellow arrow). H&E stain, magnification 100×.

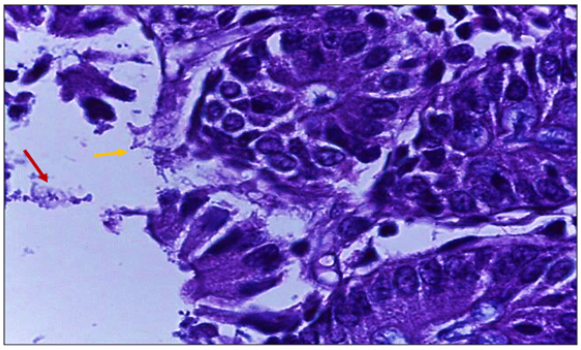

Figure 2. Histological section of the cecum from a rat infected with *Entamoeba histolytica* at 21 days post-infection. The image

shows the presence of trophozoites (red arrow) and destruction of the epithelial layer (yellow arrow). H&E stain, magnification 100x.

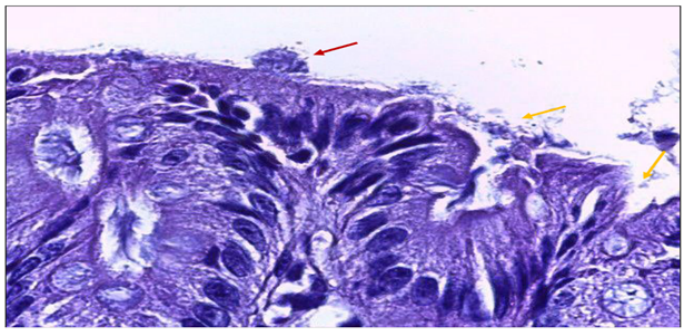

Figure 3. Histological section of the cecum from a rat infected with *Entamoeba* moshkovskii at 21 days post-infection. The image shows trophozoites on the mucosal surface (red arrow) and mild epithelial damage (yellow arrow). H&E stain, magnification 100x.

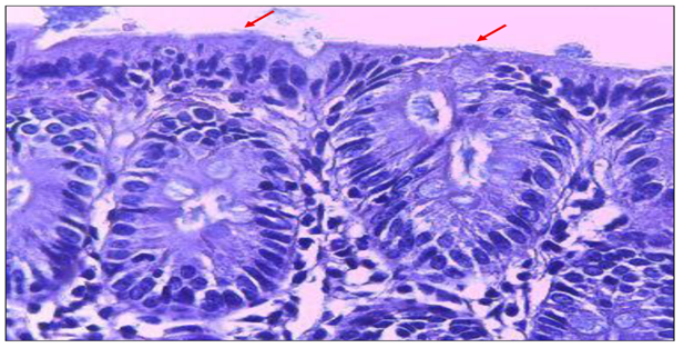

Figure 4. Histological section of the cecum from a control rat showing normal tissue architecture with an intact epithelial layer (red arrow). H&E stain, magnification 100×.

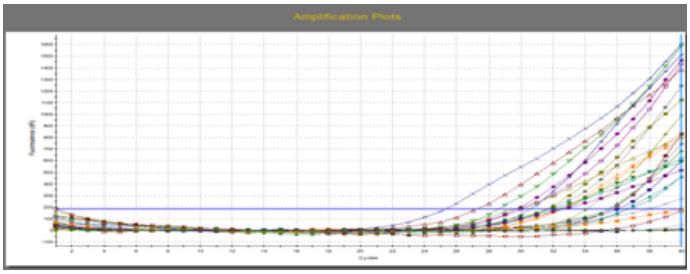

Figure 5. Amplification plot of CP, AP, GL and Act across four samples along with the negative control using SYBER green chemistry.

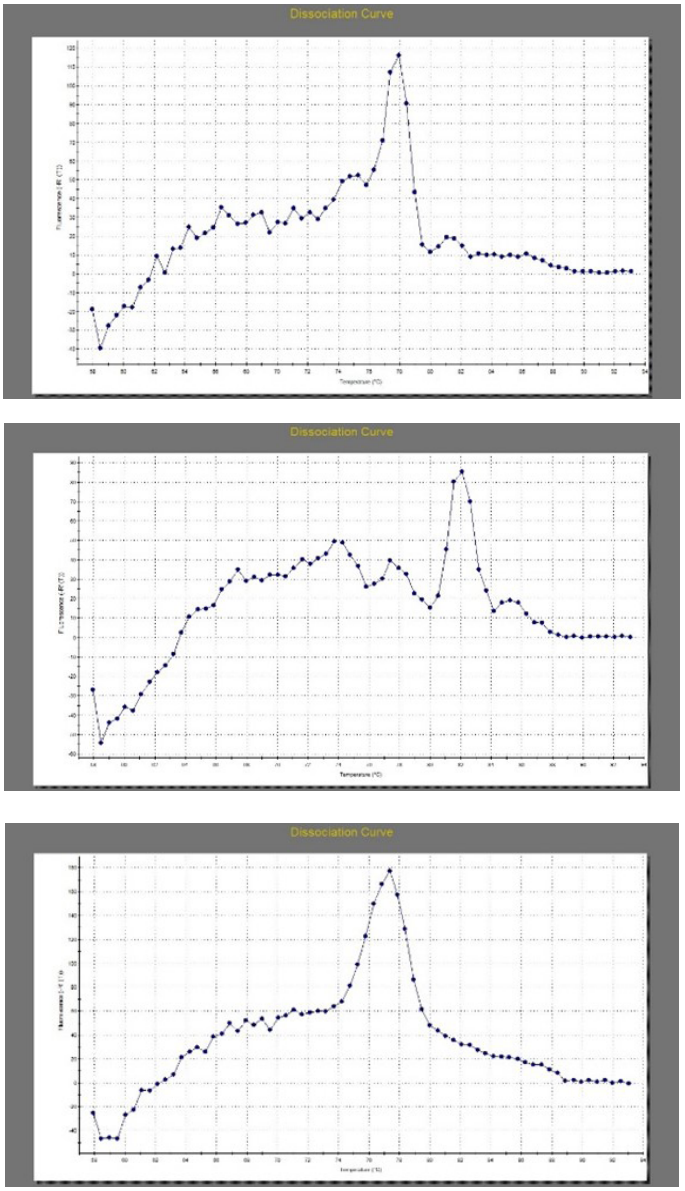

Figure 6. Melting Curve Profiles of Target Genes in Real-Time PCR Assay: A: the melting curve of AP, B: the melting curve of GAL. C: the melting curve of CP in several samples. A single peak representing the specific binding of SYBER green dye for the genes of interest.

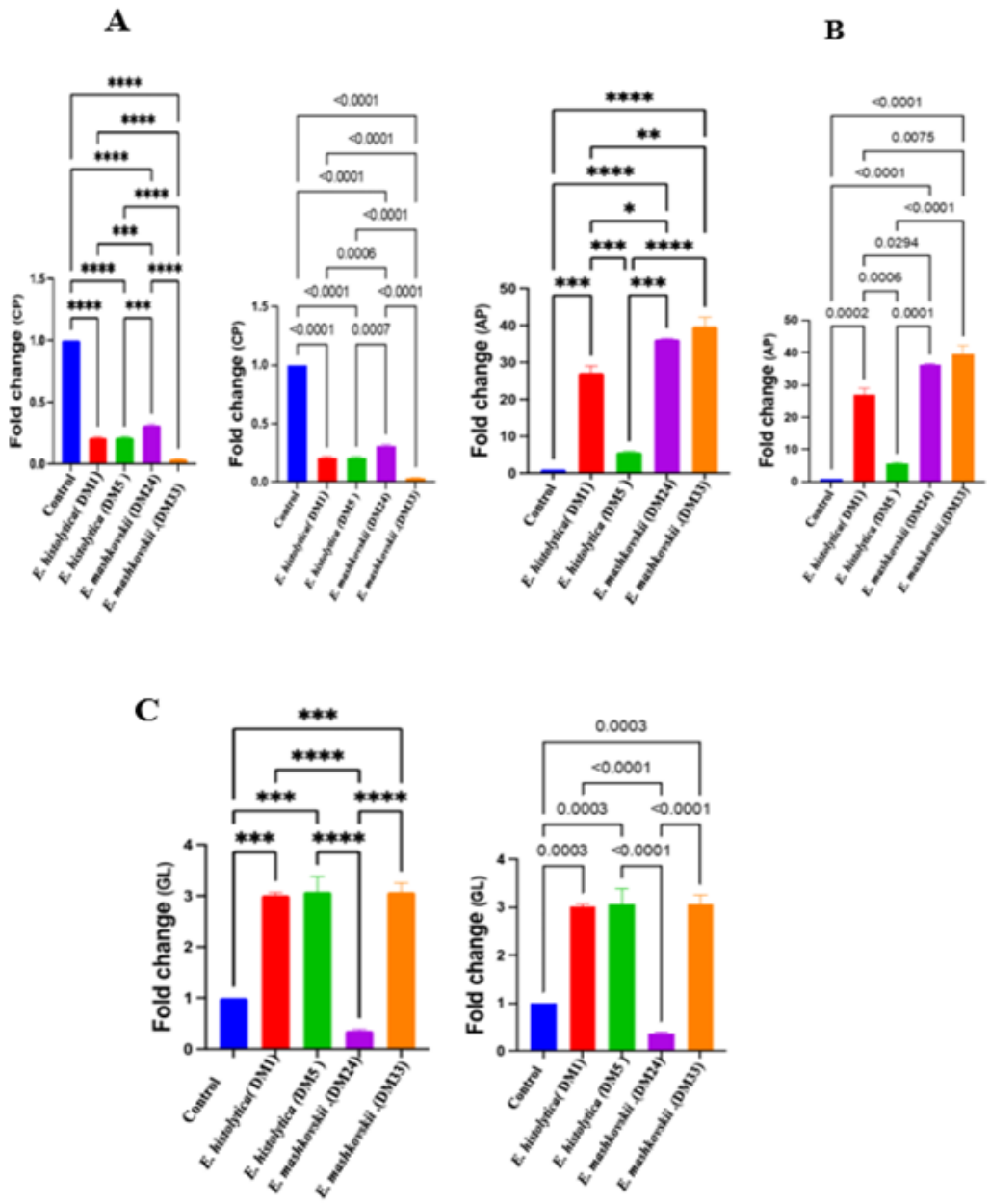

Figure 7. expression levels of A-cysteine protease (CP), B- (amoebapore (AP), and C-Gal/GalNAc lectin (GL) in the strain of *Entamoeba* spp. following experimental infection of the rat cecum.

5. CONCLUSION

This study demonstrated clear differences in pathogenicity between *E. histolytica* and *E. moshkovskii. Entamoeba* strains differ in their expression of key virulence genes, leading to variations in pathogenicity. For example, the consistently low expression of the CP gene suggests reduced tissue invasion, while differences in GAL and AP expression indicate strain-specific capacities for adhesion and cytolysis, ultimately influencing the severity of disease. Further studies should explore the full transcriptomic and proteomic profiles of both *E. histolytica* and *E. moshkovskii* under different host and environmental conditions.

REFERENCES

Al-Abodi, H. R. J., Al-Mayali, H. M. H., & Ali, M. J. (2015). Molecular study of the most important virulence factor of *Entamoeba histolytica* in Diwaniyah province. *International Journal of Advanced Research, 3*(8), 231-236.

Al-Hilfi, A. A. (2020). *Histological and diagnostic study of amoebiasis in Basrah governorate* (pp. 1-178). PhD Thesis, College of Education, University of Basra.

Aledani, A. H. E., Khudhair, N. A., & Alrafas, H. R. (2020). Effect of different methods of anesthesia on physiobiochemical parameters in laboratory male rats. *Basrah Journal of*

Veterinary Research, 19(1), 206-214.

Andrä, J., Herbst, R., & Leippe, M. (2003). Amoebapores, archaic effector peptides of protozoan origin, are discharged into phagosomes and kill bacteria by permeabilizing their membranes. *Developmental & Comparative Immunology, 27*(4), 291-304.

Bancroft, J. D., & Gamble, M. (2008). *Theory and Practice of Histological Techniques* (6th ed.).

Bansal, D., Ave, P., Kerneis, S., Frileux, P., Boché, O., Baglin, A. C., Dubost, G., Leguern, A. S., Prevost, M. C., Bracha, R., Mirelman, D., & Guillén, N. (2009). *E. histolytica* pathogenesis: low levels of Gal/GalNAc lectin and amoebapore A expression do not prevent tissue invasion. *PLoS Pathogens, 5*(5), e1000434.

Bracha, R., & Mirelman, D. (1983). Adherence and ingestion of Escherichia coli serotype 055 by trophozoites of Entamoeba histolytica. *Infection and immunity, 40*(3), 882-887.

Burgess, S. L., Gilchrist, C. A., Lynn, T. C., & Petri, W. A. (2017). Parasitic protozoa and interactions with the host intestinal microbiota. *Infection and immunity, 85*(8), e00101-17.

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.

Dolabella, S. S., Serrano-Luna, J., Navarro-García, F., Cerritos, R., Ximénez, C., Galván-Moroyoqui, J. M., Silva, E. F., Tsutsumi, V., & Shibayama, M. (2012). Amoebic liver abscess production by *Entamoeba* dispar. *Journal of Hematology, 11*(1), 107.

Frederick, J. R., & Petri, W. A. Jr. (2005). Roles for the galactose/N-acetyl galactosamine-binding lectin of *Entamoeba*in parasite virulence and differentiation. *Glycobiology, 15*, 53-59.

González-Rivas, E., Nieves-Ramírez, M., Magaña, U., Morán, P., Rojas-Velázquez, L., Hernández, E., ... & Ximénez, C. (2020). Differential pathogenic gene expression of E. histolytica in patients with different clinical forms of amoebiasis. *Microorganisms, 8*(10), 1556.

Haque, R., Huston, C. D., Hughes, M., Houpt, E., & Petri Jr, W. A. (2003). Amebiasis. *New England journal of medicine, 348*(16), 1565-1573.

Leippe, M. (1997). Amoebapores. *Parasitology Today, 13*(5), 178–183.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2– ΔΔCT method. *Methods, 25*(4), 402-408.

Lohia, A. (2003). The cell cycle of *Entamoeba histolytica*. *Molecular and cellular biochemistry, 253*(1), 217-222.

Mortimer, L., & Chadee, K. (2010). The immunopathogenesis of *Entamoeba histolytica*. *Experimental parasitology, 126*(3), 366-380.

Nagaraja, S., & Ankri, S. (2018). Utilization of different omic approaches to unravel stress response mechanisms in the parasite *Entamoeba histolytica*. *Frontiers in Cellular and Infection Microbiology, 8*, Article 19

Ohnishi, K., Kato, Y., Imamura, A., Fukayama, M., Tsunoda, T., Sakaue, Y., ... & Sagara, H. (2004). Present characteristics of symptomatic Entamoeba histolytica infection in the big cities of Japan. *Epidemiology & Infection, 132*(1), 57-60.

Petri, W. A. Jr., Haque, R., & Mann, B. J. (2002). The bittersweet interface of parasite and host: lectin carbohydrate interactions during human invasion by the parasite *Entamoeba histolytica*. *Annual Review of Microbiology, 56*, 39–64.

Que, X., & Reed, S. L. (2000). Cysteine proteinases and the pathogenesis of amebiasis. *Clinical microbiology reviews, 13*(2), 196-206.

Ravdin, J. I., Nash, T. E., & Petri, W. A. Jr. (2003). *Entamoeba histolytica* and amebiasis. In D. L. Kasper *et al.* (Eds.), *Harrison's Principles of Internal Medicine* (16th ed., pp. 983–985).

Royer, T. L., & Petri Jr., W. A. (2014). Waterborne parasites *Entamoeba*. In *Encyclopedia of Food Microbiology* (2nd ed., pp. 782-786). Academic Press.

Royer, T. L., & Petri Jr., W. A. (2014). *Waterborne Parasites | Entamoeba. In Encyclopedia of Food Microbiology* (Second Edition, pp. 782-786). Academic Press.

Ryan, K. J. (2017). Pathogenesis and Diagnosis of Parasitic Infection. In: Ryan KJ, (Ed.). *Sherris Medical Microbiology* (7th ed.). McGraw-Hill Education

Saha, A., Gaurav, A. K., Bhattacharya, S., & Bhattacharya, A. (2015). Molecular basis of pathogenesis in amoebiasis. *Current Clinical Microbiology Reports, 2*(4), 143-154.

Vicente, J.B., Ehrenkaufer, G.M., Saraiva, L.M., Teixeira, M., & Singh, U. (2009). *Entamoeba histolytica* modulates a complex repertoire of novel genes in response to oxidative and nitrosative stresses: Implications for amebic pathogenesis. *Cellular Microbiology, 11*(1), 51–69.

WHO (PAN American Health Organization). (1997). UNESCO. Expert Consultation on Amoebiasis. WHO Wkly. Epidem. Rec., 72, 97–100.

Ximénez, C., González, E., Nieves, M., Magaña, U., Morán, P., & Gudiño-Zayas, M. (2017). Differential expression of pathogenic genes of *Entamoeba histolytica* vs E. dispar. *PLOS ONE, 12*(8), e0181962.