

Molecular Detection of Pathogenic *Entamoeba histolytica* based on Amoebapore C Gene in Fecal Samples of Camels from Babylon Province, Iraq

Qassim Jawad Ameer¹, Nawras Abdul Bari Madlo¹

¹Lecturer, Branch of Parasitology, Collage of Veterinary Medicine, University of Al-Qasim Green

Abstract

The attention in the current study was paid to the identification of pathogenic *Entamoebahistolytica* (PEH) using molecular techniques targeted the amoebapore C (AP-C) gene in fecal samples of camels from Babylon province, Iraq. Fifty, 50 camels, fecal samples were collected and analyzed in a laboratory using a real-time polymerase chain reaction (RT-PCR) method to detect the amplification of the AP-C gene. The results demonstrated that 28 (56%) out of 50 samples were positive for the presence of the AP-C gene. The findings showed high efficiency rate (99.7%) of the current used technique in detecting the gene in the fecal samples of camels. The present work provides interesting data about the current presence of the pathogenic *Entamoebahistolytica* in the feces of the tested camels from Babylon Province, Iraq.

Keywords: Amoebapore C, camels, feces, pathogenic *Entamoebahistolytica*, RT-qPCR.

Introduction

E. histolytica trophozoites have some unique pathogenic abilities, and on-contact eukaryotic-cell lysing (OCECL) is considered as an important activity provided via the presence of amoebapores (Aps). Three pore-forming peptides of the AP family are included of which AP-A, AP-B, and AP-C⁹. The mechanistic approaches regarding the action of the APs are recognized by inducing OCECL via the insertion of APs into the bacterial or eukaryotic cell (EC) membranes and subsequent formation of effective lysing pores. For evaluation and confirmation purposes, it has been found that adding purified APs to ECs induced apoptosis after the occurrence of cell necrosis¹. Moreover, low trophozoitic lysing activities were detected after antisense-induced inhibition of the AP-A expression was performed. In addition, trophozoite induced liver abscesses in hamster models were recognized to be occurred in participation with low expression levels of the APs but in less virulence activities^(2,8,5). Furthermore, no expression of the AP-A gene was detected after insertion of specific transcription-silencing plasmids into the trophozoites of the *E. histolytica* trophozoites was done (6). These techniques, antisense inhibition or transcriptional silencing, provide valuable paths to determine the pathogenicity levels of *E. histolytica*.

The attention in the current study was paid to the identification of pathogenic *Entamoebahistolytica* (PEH) using molecular techniques targeted the amoebapore C (AP-C) gene in fecal samples of camels from Babylon province, Iraq.

Materials and Method

Sampling

Fifty, 50 camels, fecal samples were collected from various regions of Babylon province, Iraq. The sampling followed aseptic procedures. The samples were transported to a Lab using plastic zip logs placed in an ice box.

Extraction of genomic DNA

The extraction of the genomic DNA (gDNA) was done using Stool DNA extraction Kit (Bioneer, Korea) with considering the protocol of the kit as a guide for performing the DNA extraction. Then, the gDNA was scanned, using a NanoDrop, for its quality and quantity. The DNA was stored in a deep freezer (20°C) waiting for the next molecular identification steps.

Real-time PCR

The RT-qPCR technique was initiated to detect the

amplification, as virulence indicators of the *E.histolytica*, of the AP-C gene using specific primers and a probe, table 1, which were NCBI-GenBank- and primer3 plus-based designed and registered in the NCBI GenBank under the ID No.: X76903.1. Then, the primers were ordered from Bioneer Company, Korea.

Table 1: the AP-C primers and the probe

	Sequence (5'-3')		Amplicon
Primer	F	CCTGTTTGTACATCACTTGTGG	113bp
	R	TCAACAAGACCATCTGCTTAGC	
Probe	FAM- TGATTTAGTCCTTGGTGGAGCAGTTG -BHQ1		

The reaction components of the RT-qPCR amplification included the use of AccuPower® DualStar™ qPCR PreMix kit (Bioneer, Korea) and preparation of the master mix depending on the company instructions was performed using 20µl of a total volume for the reaction that contained 2µl gDNA, 1µl (10pmol) for each direction of the AP-C primer, 2.5µl (20pmol) of the probe, and the total volume was reached using 13.5µl of nuclease-free water. The RT-qPCR technique was generated using MiniOpticon Real-Time PCR system under the thermocycler conditions of one-cycled initial denaturation under 95°C for 5min and 45-cycled denaturation and annealing/extension under 95°C and 60°C, respectively, for 30s and 1min, respectively.

Results

The results demonstrated that 28 (56%) out of 50 samples were positive for the presence of the AP-C gene. The CT values were ranged from 22.26 to 34.89, figure 1.

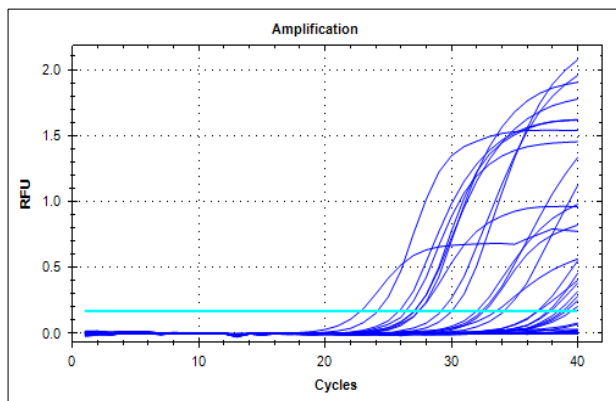


Figure 1: The amplification plot of the RT-PCR that demonstrates the AP-C gene positive amplification in Entamoebahistolytica. No amplification is shown under the threshold line.

The TaqMan probe (FAM) used in the present work showed high efficiency rate (99.7%) of the current used technique in detecting the gene in the fecal samples of camels. This efficiency was recognized using a standard curve involving DNA serial dilution of the positive samples, figure 2.

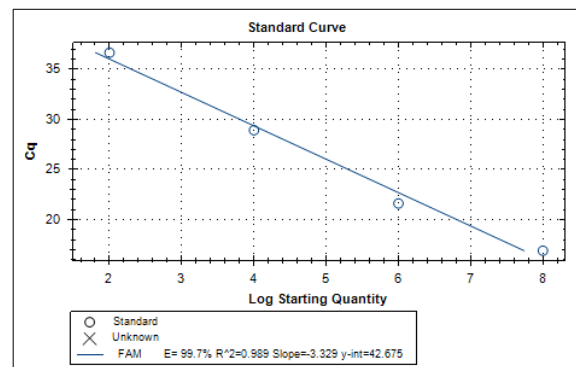


Figure 2: Standard curve of RT-PCR that shows high efficiency (99.7%) of the TaqMan probe for the serial dilution of the DNA positive samples.

Discussion

APs are well-known proteins recognized for their activities in the formation of eukaryotic cell membrane pores via establishing themselves in those membranes and generating oligomers of ionic-channel peptide-peptide interactions resulting in a lysis process of the host cells. These actions are similar to that from the natural killers (NKs) granulysin proteins and the cytotoxic lymphocyte granules (7, 1,4).

The results showed the amplification of the AP-C gene using the RT-PCR method employed in the current study, and this indicates that the *E.histolytica* positive samples were all pathogenic that induce host cell membrane lysis due to the presence of the AP-C gene product. The finding agrees with¹⁰ who detected

that full pathogenicity of *E.histolytica* is maintained by the expression of the AP genes suggesting important role of the APs in the host-cell damages upon contact by trophozoites. The critical needs for *E.histolytica* to induce a severe disease are fulfilled by the expression of the AP genes leading to promote host-cell destruction via the activities of the AP proteins inserted the membranes of those host cells. Studies have recognized this importance in the virulence of *E.histolytica* as those APs are normally present in granules located in the trophozoite cytoplasmic and projected outside those granules and the membrane upon initiating a contact with host-cell membrane.

Conclusion

In conclusion, the present work provides interesting data about the current presence of the pathogenic *Entamoebahistolytica* in the feces of the tested camels from Babylon Province, Iraq.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Collage of Veterinary Medicine, University of Al-Qasim Green and all experiments were carried out in accordance with approved guidelines.

References

1. Andrä J , Herbst R, Leippe M. ‘Amoebapores, archaic effector peptides of protozoan origin, are discharged into phagosomes and kill bacteria by permeabilizing their membranes.’, *Developmental and comparative immunology*, 2003;27(4): 291–304.
2. Bracha R. ‘Antisense inhibition of amoebapore expression in *Entamoeba histolytica* causes a decrease in amoebic virulence.’, *Molecular microbiology*, 1999;34(3): 463–72.
3. Espinosa-Cantellano M, Martínez-Palomo A. ‘Pathogenesis of intestinal amebiasis: from molecules to disease.’, *Clinical microbiology reviews*. American Society for Microbiology (ASM), 2000;13(2): 318–31.
4. Haque R. ‘Diagnosis of amebic liver abscess and amebic colitis by detection of *Entamoeba histolytica* DNA in blood, urine, and saliva by a real-time PCR assay.’, *Journal of clinical microbiology*. American Society for Microbiology (ASM), 2010;48(8): 2798–801.
5. LEIPPE M. ‘Ancient weapons: the three-dimensional structure of amoebapore A’, *Trends in Parasitology*, 2005;21(1): 5–7.
6. Tanyuksel M , Petri WA, Jr. ‘Laboratory diagnosis of amebiasis.’, *Clinical microbiology reviews*. American Society for Microbiology (ASM), 2003;16(4): 713–29.
7. Ximénez C. ‘Differential expression of pathogenic genes of *Entamoeba histolytica* vs *E. dispar* in a model of infection using human liver tissue explants.’, *PloS one*. Public Library of Science, 2017;12(8): e0181962.
8. Zhai Y, Saier MH. ‘The amoebapore superfamily.’, *Biochimica et biophysica acta*, 2000;1469(2): 87–99.
9. Zhang X , Zhang Z , Alexander D , Bracha R , Mirelman D , Stanley Samuel L, et al. ‘Expression of amoebapores is required for full expression of *Entamoeba histolytica* virulence in amebic liver abscess but is not necessary for the induction of inflammation or tissue damage in amebic colitis.’, *Infection and immunity*. American Society for Microbiology (ASM), 2004;72(2):678–83.
10. Zhang X , Zhang Z , Alexander D , Bracha R , Mirelman D , Stanley Samuel L , et al. ‘Expression of Amoebapores Is Required for Full Expression of *Entamoeba histolytica* Virulence in Amebic Liver Abscess but Is Not Necessary for the Induction of Inflammation or Tissue Damage in Amebic Colitis’, *Infection and Immunity*. American Society for Microbiology (ASM), 2004;72(2): 678.