DIAGNOSIS OF AMEBIC LIVER ABSCESS BY A COMMERCIAL MULTIPLEX STOOL AMPLIFICATION ASSAY. A POTENTIALLY USEFUL ALTERNATIVE IN LIMITED RESOURCE SETTINGS

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INTRODUCTION

Presentation of Amoebic Liver Abscess (ALA) caused by *Entamoeba histolytica* often mimics that of Pyogenic Liver Abscess (PLA) rendering both conditions indistinguishable clinically and by imaging techniques. Indeed, computed tomography, magnetic resonance and ultrasonography usually demonstrate the presence of space occupying lesions consistent with abscesses but do not differentiate between ALA and PLA. Current tools of diagnosis are not sensitive and/or specific enough and rely on the examination of the aspirated hepatic pus by special stains, cultures and cytology. However, trophozoites are seen in less than 25% of the cases. Amoebic serology is less useful, especially in patients that have been previously exposed. Although various in-house PCR protocols have successfully been applied in ALA pus specimens, they are expensive, exhibit considerable performance differences, lack proper standardization, and require long hands-on time, making them unsuitable for countries with limited resources. Therefore, there is a need for new, rapid and accurate microbiological methods for diagnosis of ALA. We here report our recent experience with the successful use of a commercial multiplex nested polymerase chain reaction (PCR) assay (designed for stools) in the diagnosis of three cases of ALA.

MATERIALS / METHODS

- Three Latin American patients are reported.

PATIENT 1

- 59-year-old male, originally from Venezuela, visiting the United States, who presented on 01/13/2022 with chills, fatigue, and 10 days of watery, non-bloody diarrhea (3-4 bowel movements per day)
- Physical examination: Afebrile, tenderness to deep palpation in epigastrium and right upper quadrant of the abdomen.
- Epidemiology: Lives in Venezuela. No previous diagnosis of amebiasis
- Laboratory: 01/13/2022 : WBC 24, 300 K/ul . Hgb 11.0 g/dL . ALT 60 U/L. AST 55 U/L . ALK Phos 548 U/L. Bilirubin 1.5 mg/dL. Albumin 1.7 g/dL
- CT scan of the abdomen/pelvis with IV contrast without oral contrast shows inflammatory large cystic lesion in the left lobe of the liver measuring 15.7x10.8x9.7 cm. There is also thrombosis of the left portal vein.- [FIGURE A]
- Started on Piperacillin/Tazobactam
- IVR consulted

PATIENT 2

- 43-year-old female, originally from Cuba, who presented on 03/03/2022 with dyspnea, epigastric pain and fever, previously diagnosed with acute cholecystitis- unresponsive to antibiotics.
- Physical examination: Febrile to 101º F , tenderness to deep palpation in epigastrium and right upper quadrant of the abdomen.
- Epidemiology: Moved from Cuba to Uruguay two years prior to migrating to the United States. Traveled by land from Uruguay to Peru, Colombia, Central America, Mexico and then the United States in November 2021.
- Laboratory: 03/03/2022: WBC 23, 400 K/ul . Hgb 9.6 g/dL . Plt 635,000 K/ul . ALT 28 U/L . AST 18 U/L. Alk Phos 337 U/L . Bilirubin 0.5 mg/dL. Albumin 2.1 g/dL.
- CT of the abdomen/pelvis with IV/without po contrast: Complex cystic lesion in the right lobe of the liver measuring 13.3x10.7x12.1 cm. Elevation of the right hemidiaphragm, right basilar atelectasis. [FIGURE B]
- MRI Abd with contrast: 14 cm thick walled, rim enhancing complex inflammatory collection within the right and left hepatic lobes, compatible with an abscess. [FIGURE C]
- IVR consulted.

PATIENT 3

- 57-year-old male who lives in Miami, Florida presented on 05/11/2022 with fever and upper abdominal pain.
- Physical examination: Tenderness to deep palpation in epigastrium and right upper quadrant of the abdomen.
- Epidemiology: Lives in Miami, Florida. Traveled to Mexico - June 2021. Had diarrhea treated with antibiotics, Visited Colombia - March 2022. Had diarrhea. Positive stools for *Shigella*. Treated with antibiotics
- Colonoscopy April 2022 showing cryptitis and active colitis.
- Laboratory: 05/11/2022: WBC 8,700 K/ul . Hgb 13.9 g/dL. Hct 41.6 . Plt 213,000 K/ul . ALT 29 U/L . AST 25 U/L Alk Phos 78 U/L . Bilirubin 0.7 mg/dL. Albumin 2.1 g/dL.
- MRI of the abdomen with/without contrast shows a 5.2x3.6 cm inflammatory lesion in the right lobe of the liver,
- corresponding to an abscess [FIGURES D & E]
- IVR consulted.

IMAGES

FIGURE A

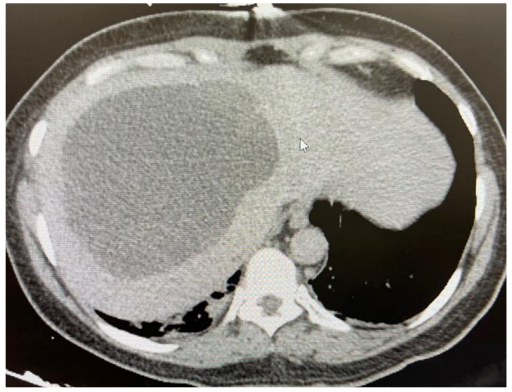

FIGURE B

FIGURE C

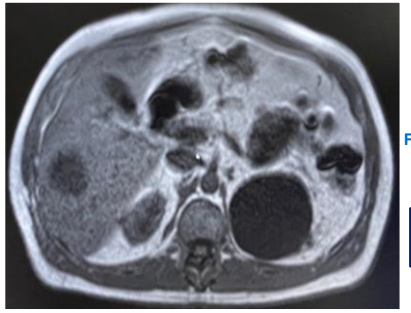

FIGURE D

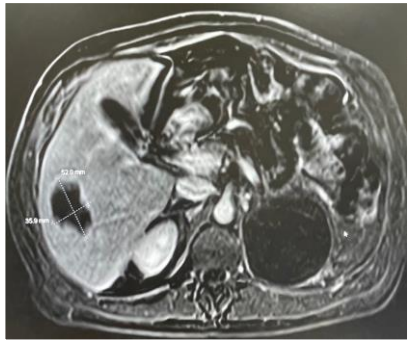

FIGURE E

RESULTS

DIAGNOSTIC TEST	PATIENT 1	PATIENT 2	PATIENT 3
PCR **Biofire Film Array Multiplex G.I.® Aspirated Hepatic Pus E. histolytica	POSITIVE (180 mL) (01/14/2022)	POSITIVE (800 mL) (03/14/2022)	POSITIVE (60 mL) (05/13/2022)
*Amoebic Serology	REACTIVE (01/18/2022)	REACTIVE (03/17/2022)	REACTIVE (05/16/2022)
PCR **Biofire Film Array Multiplex G.I. ® Stool samples E. histolytica	REACTIVE (01/18/2022)	NON-REACTIVE (03/06/2022	REACTIVE (05/11/2022)

Collected Date: 1/18/2022
Collected Time: 15:05 EST

Procedure		Units	Reference Range
Campylobacter-PCR	Not Detected		[Not Detected]
Plesiomonas shigelloides-PCR	Not Detected		[Not Detected]
Salmonella-PCR	Not Detected		[Not Detected]
Vibrio-PCR	Not Detected		[Not Detected]
Vibrio cholerae-PCR	Not Detected		[Not Detected]
Yersinia enterocolitica-PCR	Not Detected		[Not Detected]
Adenovirus F 40/41-PCR	Not Detected		[Not Detected]
Astrovirus-PCR	Not Detected		[Not Detected]
Cryptosporidium-PCR	Not Detected		[Not Detected]
Cyclospora cayetanensis-PCR	Not Detected		[Not Detected]
Entamoeba histolytica-PCR	Detected		[Not Detected]
Giardia lamblia-PCR	Not Detected		[Not Detected]
Norovirus GI/GII-PCR	Not Detected		[Not Detected]
Rotavirus A-PCR	Not Detected		[Not Detected]
Sapovirus-PCR	Not Detected		[Not Detected]
Enterotoxigenic E coli-PCR	Not Detected		[Not Detected]
Shiga-like toxin producing E coli-PCR	Not Detected		[Not Detected]
Shigella/Enteroinvasive E coli-PCR	Not Detected		[Not Detected]
Enteroaggregative Ecoli-PCR	Not Detected		[Not Detected]
Enteropathogenic E coli-PCR	Not Detected		[Not Detected]

Result Comments
1: Entamoeba histolytica-PCR

*Biofire Film Array G.I. panel® (Biomerieux)

CONCLUSIONS

Three adult patients with positive PCR for *E. histolytica* from aspirated hepatic abscesses are reported. Analysis of pus done with a commercially available multiplex, nested PCR assay, designed for diagnosis of enteric pathogens in stools was performed. Results obtained same day of aspiration of the liver, allowing confirmation of diagnosis and choosing specific therapy for single pathogens. Even though two of our three cases had positive antigen in stools for *E. histolytica*, most patients with extra intestinal amoebiasis, especially ALA, do not have concurrent amoebic colitis and the parasite may no longer be present in stools, as illustrated by Patient 2. Studies need to be performed in areas of the world with higher incidence of ALA, such as Latin America, Africa, Southeast Asia or the Indian Subcontinent. This type of analysis provides theoretical and practical advantages for diagnosis of ALA, such as simplicity, rapid processing time, capacity to exclude other pathogens. Further testing is necessary to validate the use in liver abscess diagnosis, defining false positive and false negative rates.

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