# Differentiation Between Two Isolates Of Entamoeba histolytica Isolated From Different Clinical cases After Intraceacal Inoculation In Hamsters

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## ABSTRACT:

**BACKGROUND:**

The aim of this study is to differentiate between virulence of two isolates ,one was isolated from severe clinical symptoms of amebiasis isolate (A) the other was from asymptomatic carriers isolate (B).

**METHODS :**

12 male hamsters were employed in this study , they were divided into 3 groups . 1st group (5 hamsters), inoculated intracecally with isolate isolate (A) from human case of severe amebiasis . 2nd group (5 hamsters) inculated with isolate (B) from asymptomatic carriers .3rd group (2 hamsters)inoculated with saline solution as control group .

**RESULTS :**

1st group of hamsters showed a flask shaped ulcer with sever inflammation of cecum with liver abscesses , after four weeks of infection The 2nd group showed only mild inflammation of cecum without ulceration and liver abscess. While 3rd group were normal control group.

**CONCLUTION:**

Our study may be Considered as a step for Characterization of Entamoeba histolytica that cause the invasive intestinal and extraintestinal amebiasis and E.dispar an intestinal commensal parasit in Iraq.


**KEY WORDS:** Entamoeba histolytica , Virulence, Amebiasis .

## INTRODUCTION :

Amoebiasis is due to invasion of the colon mucosa by E-histolytica, it is an important parastic disease in human , have a variety of affects ,(2) .

E-histolytica trophzoites first colonize the human of larg intestine in 80% to 90 % of cases E-histolytica remains in the lumen and there are either no clinical symptoms or patients complain of mild gastro intestinal disconfort (3) .

In 16% of cases the amoebae become invasive adhering to and digesting the wall of intestine forming flask shaped ulcer (4). E.histolytica has recently been reclassified as two distinct but morphologically identical intestinal parasites, E.histolytica causative organism of invasive amebiasis and E.dispar non pathogenic intestinal commensal parasite (5) , this reclassification stemmed from a cumulative Clinical , Biochemical , Immunologic and genetic data (6).Infection with E.dispar is approximatly 10 times more common E.dispar be revivad for the beign specie. In 1997, the world health organization (WHO) formally accepted this redefinition (7).


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## MATERIAL AND METHODS :

Two isolates of Entamoeba histolytica were used from stock cultures of morphologically identical E.histolytica cultivated and maintained on Cleveland and Collier medium at 37°C .The amoebae were chosen carfully from patients with symptomatic dysentry and asymptomatic carrier . 0.05 ml of culture suspention adjusted to contain about 500,000 trophozoites were inoculated interacecally in hamster under ether anaesthesia. 0.05 ml of saline solution were used for inoculation control group of hamster .12 male hamster mescaricetus auratus were used as experimental animals their wieght range between 100-120 gm .

**Groups of animals :**

**Group I** 5 animals inoculated with 0.05 ml suspention from positive amoebae culture isolated from acute case of amoebic dysentery ( isolate A).

**Group II** 5 animals inoculated with 0.05 ml from positive amoebae culture isolated from asymptomatic cases ( isolate B).

**Group III** 2 animals inoculated with 0.05 ml saline solution as control group . Hamster were killed with an overdose of ether at the end of experiment which was 4 weeks post inoculation . Direct wet smear of hamster stool specimens were

examined before and after inoculation . The caecum was removed and gently washed with saline , it was cut open with scissors, the mucosa was exposed , portions of caecum which were regarded as grossly damaged was taken , liver was removed and examined to absorve the number and severity of abscess . Specimens were taken from theses organs , fixed with 10% formalin embedded in paraffin sectioned and stained with eosin and hematoxyline for histophathological studies .

## RESULTS :

***Group I Cecum .*** Histopathological studies showed a flask shaped ulceration with infiltration of acute and chronic inflammatory cells Fig (1), inflammatory exudate is occupying the lumen mainly , trophozoite are seen within the lumenal exudate Fig (1,2). The amoebae pentrates the mucosal epithelium as a role of polymorphic leucocytes in the production of amoebic lesions .

***Group I Hepatic Lesions:*** (4 of 5) infected hamsters showed lesions . The lesions were focal , well demarcated creamy white areas scattered irrgularly , lesions were mostly subcapsular .

Microscopic wet smear examination of stool specimens shows positive trophozoite of E.histolytica. The body weight loss was 15 gm table (1).

***Group II Cecum .*** Histopathological studies showed mild inflammation , inflammatory cells include Lymphocytes plasma cells and neutrophil without ulceration .

***Group II Hepatic Section*** Hepatic section was normal No Lesions were seen . Stool examination was positive for trophozoite with increase in body weight table (1). ***Group III*** Stool examination showed negative for E.histolytica . Body weight showed increased ( 10 gm ) and normal grossly apperance of Cecum and liver .

## DISCUSSION :

From above observations it seems that the experimental model showed variation in the symptoms of the infected animals due to the virulence of the strain [7]. The major Clinical syndromes that result from infection with isolate (A) include chronic invasive colitis with liver abscesses which suggest that the isolates was highly virulent and invasive (8,9) , in contrast isolate (B) which was isolated from asymtomatic carriers showed only mild inflammation of cecum without ulceration of the liver [10] . Our results are confirmative to other recent study done on the same isolates , when isolate (A) was highly virulent produced multible liver abscesses and acute inflammation while isolate (B) showed mild inflammation only ( a virulent ) when its inoculated interahepatically in hamster [11] . These results are also confirmative to other study done on the same isolates using electrophorysis analysis, was showed different electrophorysis patterns [12]. The presence of different isonenzyme varient groups revealed that there are certain isoenzyme patterns for each isolate [13,14] .

**conculation** is reached that there are two strains of morphologically identical E.histolytica that cause invasive intestinal and extraintestinal amebiasis and E.dispar that has never been shown to cause human disease [12,13] .

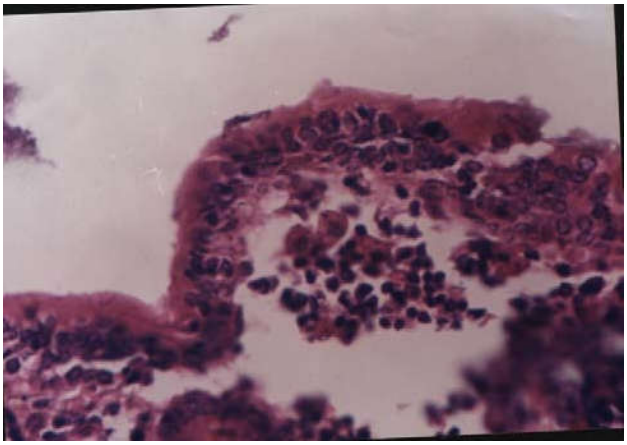


**Figure 1 : Cecum section showed a flask shaped ulcer, trophazoites are seen after intraceacal inoculation of isolate (A) (x600)**

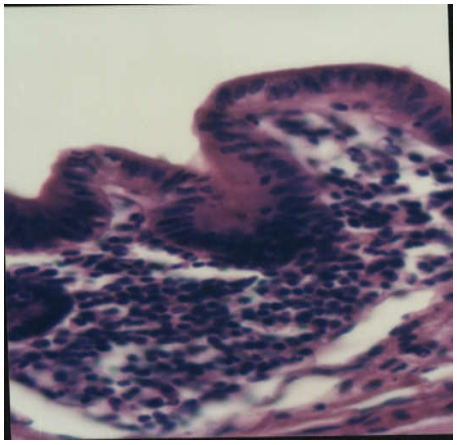


**Figure 2 : Cecum section showed ulceration with acute and chronic inflammatory cells after intraceacal inoculation of isolate (A) (x600).**

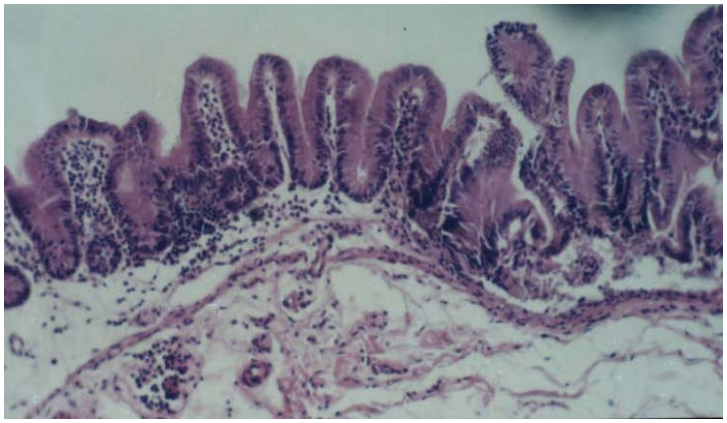


**Figure 3: Cecum section showed mild inflammatory cell , lymphocyte plasma cell and neurtrophile after intraceacal inoculation with isolate (B) (x400)**

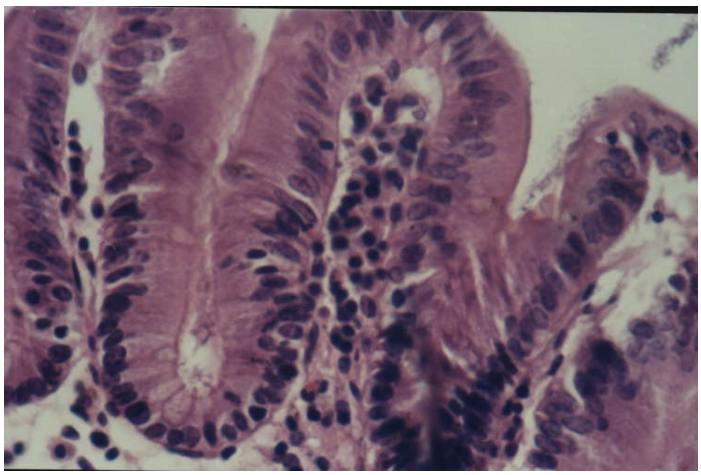


**Figure 4 : Cecum section showed mild inflammatory cells after intraceacal inoculation with isolate (B) (x600)**

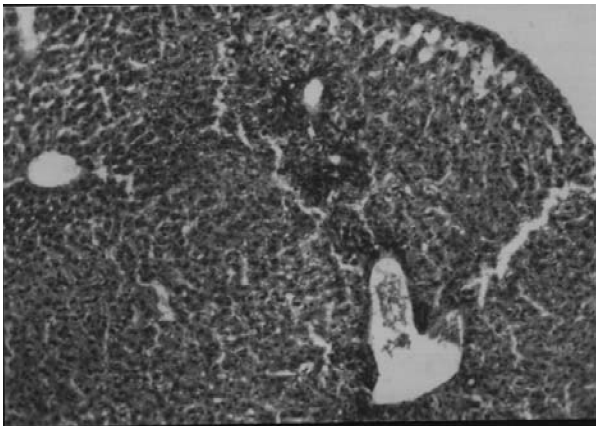
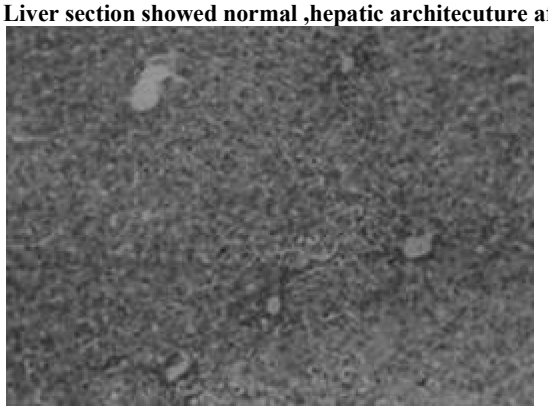


**Figure 5 : Liver section showed Liver abscess after intraceacal inoculation with isolate (A) (x600)**

**Figure 6 : Liver section showed normal ,hepatic architecuture after**



**intraceacal inoculation with isolate (B) (x600)**

| Animal group | Stool examination | Change in the body weight | Cecum | Liver |
|---|---|---|---|---|
| Group I | Positive with trophozoite | -15 gm | Severe ulceration | Many abscesses |
| Group II | Positive with trophozoite | +5 gm | Mild inflammation | No abscesses |
| Group III | negative with trophozoite | +10 gm | No intestinal Lesions | No abscesses |

**Table 1: stool examination, body weight and grossly apperance of three group of animals included in this study**

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