Culture of Entamoeba histolytica in vitro

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Abstract

Entamoeba histolytica, a world wide distribution between human and animals, with a range between 0.5 - 50% of the population in the world which harbors this parasite. The cultivation of E. histolytica was done for the first time successfully at Basrah city under this new study. A high number of trophozoite were found in culture and sub culture in vitro, with a new modified method used. There was a relationship between pH level and parasite development in culture and sub culture, further more, a high destruct in parasite development was found when used antibiotic (Erythromycin 25%) in media under study.

Introduction

The cultivation of intestinal protists has along history, not all parasites are amenable to growth in vitro (1, 2). Entamoeba histolytica most important pathogenic protists, it was first discovered in 1973 by a clinical assistant in Russia, from a young peasant patient with bloody dysentery, was passing large numbers of amoebas in his stool (3). E. histolytica is primarily of man, infected and carrier humans forming the reservoir of infection. Man is also the reservoir host for animal infections. Infection is by the ingestion of the mature cysts, and trophozoite which not survive along out side the host but cysts are relatively resistant to adverse condition (4). Arrange between 0.5 - 50% of the population world wide harbors (4) E. histolytica parasites with the higher rates of infection being under developed countries and associated with poor hygiene. There are two stages in the life cycle of E. histolytica:-

(I) Trophozoite: This form has an amoeboid appearance and is usually 15-30 μm in diameter, the organism has a single nucleus with a distinctive small central karyosome the fine granular endoplasm may contain ingested erythrocytes the nucleal chromatin is eventually distributed along the periphery of the nucleus.

(II) Cysts: Are spherical, with retractile wall, the cytoplasm contains dark staining chromatoidal bodies and 1-4 nuclei with a central karyosome and eventually distributed peripheral chromatin (3,4). Infection occurs by ingestion of cysts on fecally contaminated food or hands. The metacysts divide into four and then eight amoebae which move to the large intestine. The majority of the organisms are passed out of the body with the feces. But with larger bolus of infection, some amoebae attach to and invade the mucosal tissue forming "flask-shaped" lesions (bomb craters). The organism encysted for mitosis and is passed through with feces. Intestinal amoebiasis cannot be reliably diagnosed on clinical grounds only. Primary dependence is placed on direct microscopic in stained sections of tissue obtained at biopsy or autopsy, distinguishing more morphological differences between E. histolytica and other species of Entamoeba requires technical skill and experience (5). Without the ability to cultivate organisms few basic studies can be performed beyond morphological and pathological. Distripiton and cultivation is prerequisite for studies that require larger numbers of cells. In Basrah there was one study for cultured protozoa in vitro, it was urogenital trichomoniais among women, but no one use E. histolytica for culture invitro at Basrah city (6). The aim of this study is to investigate if E.histolytica can be cultured successfully invitro, with measure of some environmental factor which surrounded it with some attempts to found best conditions for successful culture.
Material and Method

Culture Method: First sterilization of test tube and all condition of culture done under sterilization in laminar flow.

Media: Lock- egg slant medium (L.E.S), this medium is used for culturing the E. histolytica in clinical specimens.

Preparation and Usage:
To prepare LE medium (NIH Modifications of Boeck and Drboh lav's media) (7). First prepare Locke's solution by dissolving the salt in 500ml/D.W. To prepare the egg slant, surface to eggs sterilize by flaming in 70% ethanol and break in to a graduated cylinder. Add 2 ml of eggs mixture in to Locke's solution emulsified. Add 8 ml amounts of the emulsified egg to some standard culture tube, and 13ml to others standard cultured tube and measure pH of media by pH-meter and put the tubes in slant positions. After cooling and in oven under temperature at 80 C° for cooling. After cooling the slants leaves at room temperature, and refrigerator under 4 C°, when use for culture add serum of the donkey at ratio 1:8 of medium and add rice starch small amount (which sterilized in autoclave at 37 C° for 24-27 hours which prepare from 50ml of donkeys blood were centrifugated in test tube for 3 min at 5000 rpm).The serum collected and kept in the freezer till use, and use serum of donkey instead of serum of horses and a modified procedure was used under this study.

Note: Sterilized of starch by put in the test tubes in the oven under temp, at 180 C° for one hour.

Collection of Sample:
The samples were collected from patients with amoebic dysentery from Basrah general hospital and from privet laboratory randomly. The samples were pooled and kept at room temperature until use about 48h.

Elimination:
Add about 1ml of D.W on stool samples at room temperature for about 15 min. (8). Then emulsified in saline and passed through a mesh to remove most of the larger particulates and determined the total size of sample.

Examination:
One drop of sample was examined on direct microscopic examination of the faeces and calculated the numbers of cysts (C) or Tropozoite (T). Determination can be done by the numbers of C or T in one ml of sample by use this equation:-

\[
\text{No. of (C) or (T)} \times 15.25 = \text{no of (C) or (T) in one ml sample.}
\]

Establishment of Culture:
Small amount (0.25ml) of emulsified stool sample was cultured on the LES media, small – pea –size pieces of fecal material can be added directly to the medium. It is always a good idea to include portions of the stool that appear mucoid or
bloody if these are present. In this study a duplicate culture was used with diluted antibiotics (0.25 mg of Erythromycin).

**Incubation:**
Culture tube, containing medium and rice starch, to which stool-derived material has been added, are incubated vertically at 37 °C for 48h before examination. After 48 hours were measure pH of culture media.

**Examination of Culture Media:**
A drop of sediment can be extracted from the tube for examination on microscope slide. Counted the no. of (C) or (T) as above. If no growth is observed at 48h. A blind passage should be made. Most of the liquid overlying the sediment is discarded to leave less than 1 ml in the tube. The Sediment is resuspended in the remaining fluid and transferred to a fresh culture tube with medium and rice starch. After incubation for an additional 48h the culture is reexamined as describe above. If no amoebae are seen further incubation is wanted, and this is followed by reexamination. If there are still on amoebae seen, the culture is discarded as negative.

**Isolations:**
If the culture if positive for amoeba, it is usually helpful to centrifuge the culture and divided the pellet among the recipient tubes. This can be done by chilling the culture tubes for 5 min. in an ice-waterbath, inverting several times to detach adherent amoebae and transferring the liquid phase to an empty culture tube before centrifugation. As growth improves, centrifugation is no necessary as measured inoculation<2ml can be transferred to the fresh medium contain 8 ml of media.

**Incubation:**
Use the procedure above. And measure the pH value after incubation period. Determined the best pH for survival of Trophozoite. And the period of survival of T after culture and subculture also comparing the survival of (T) in the culture which have antibiotic and culture don’t have.

**Results**
Table (1) showed the results of primary culture of the *E. histolytica* which found that the number of cyst was 457.5/ml, while the trophozoite was 152.5/ml. By the other hand, the number of cyst after subculture was 183/ml and 549/ml for trophozoite number.

<table>
<thead>
<tr>
<th>Number per ml</th>
<th>Culture</th>
<th>Sub Culture</th>
<th>Sum total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyst</td>
<td>457.5</td>
<td>183</td>
<td>640</td>
</tr>
<tr>
<td>Trophozoite</td>
<td>152.5</td>
<td>549</td>
<td>701</td>
</tr>
</tbody>
</table>

The present study founded that the pH of media was decreased with time under the experiment times. In table (2) can showed that the number of *E. histolytica* (cyst & trophozoite) from the original samples & from culture and sub cultured media under experiment with different pH.

**Table (2) The number of Cyst & Trophozoite of *E. histolytica* with different pH.**

<table>
<thead>
<tr>
<th>PH</th>
<th>Number per ml</th>
<th>Cyst</th>
<th>Trophozoite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original sample</td>
<td>762.5(7.91)</td>
<td>122(7.91)</td>
<td></td>
</tr>
<tr>
<td>Culture</td>
<td>61(5.95)</td>
<td>152(5.95)</td>
<td></td>
</tr>
<tr>
<td>Sub Culture</td>
<td>61(4.94)</td>
<td>-------</td>
<td></td>
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</tbody>
</table>
Under the present study founded that the antibiotic (Erythromycin) susceptibility at concentration of 25% resulted a high range of died *E. histolytica* both cyst and trophozoite.

**Discussion**

The amoeba responsible for such misery is the third most common cause of parasitic death in the world (9). Diagnosis of *E. histolytica* based on the demonstration of the trophozoite or the cyst in the feces. In normally formed stool, usually only cysts are found, but in diarrheic stools the trophozoite is also
seen. Activity motile organism may be seen in warm, freshly passed diarrheic faeces; but at other times the cysts may be seen more readily if a drop of iodine solution is added (10). Immunological diagnosis of amoebiasis has been advanced by the fluorescent antibody technique introduced by gold man (1954, 1960) (4). In this study we used a diphasic media L. E. for culturing E. histolytica in Basrah city under in vitro conditions, it is a simple media and have the main components for parasite's growth with a rice starch use as a carbohydrate source with serum as a source of lipids, furthermore, without any sugars because it would be metabolized rapidly by the bacteria and this would prevent the necessary bacterium - imbalance from being reached in culture. Under recent study, the pH were decreased when the culture were many timed. This may be refer that E. histolytica consumption materials in media for development & survive. By the other hand, the number of trophozoite was increase as compared with number of cysts. As contrast with many studies which pointed that number of cysts higher than trophozoite number in cultured media. This may be referring that media under study with high nutrients which make trophozoite live for long periods (10). A new protocol with results shows that by simple materials & media can E. histolytica cultured with high & new results. This can be benefit for future studying on this parasites and also can be used for classify amoeba to strain by using PCR, TLC and Column chromatography. A direct smear examined either as a wet mount or fixed and stained will usually reveal heavy in faction. Lighter infections of cysts passers be detected with concentration techniques, such as zinc sulfate flotation modern molecular techniques (Polymerase chain reaction, PCR) are also being employed in efforts to detect E. histolytica DNA in stool samples and fluid with drawn from liver abscesses. Serological procedures that have been adopted widely are the hem agglutination test and agar gel diffusion. The cause for endemic, hyperendemic, or epidemic amoebiasis in a population is to be found in its epidemiological pattern, how the agent is maintained and propagated. Therefore, studies must be made to determine whether water, food handlers, person to person contact, fifth files, or passably reservoir hosts are the responsible factors. Then practical methods must be set up to control transmission. The control of E. histolytica is essentially a question of good sanitation, improved sewage disposal, the avoidance of faecal contamination of food, and an improvement in personal hygiene.

Reference


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الخلاصة

يعتبر طفيلي الزحار الأميبي Entamoeba histolytica من الطفليات ذات الانتشار العالمي، مسبب للإصابة في الأنسان والحيوانات في العديد من دول العالم. لوحظ أن نسبةً قد تراوحت ما بين 5-50% من الأشخاص الحاملين للأمراضة في دول العالم. إن استزراع طفيلي الزحار الأميبي في ظروف مختبرية معروف على نطاق العالم ولكن نجاح زرع طفيلي الزحار الأميبي مختبريا بجانب آخر مرة في مدينة البصرة. أعداد كثيرة من طور الناشئة Trophozoites وجدت في الزرع الأولي والثاني وتم تطوير الطفيلي في pH 6.5 تحت تأثير 25% Eryromycin. هناك علاقة بين مستوى Sub culture الأولي والثاني ووجدنا تطور عالى في تطور الطفيلي عند استخدام المضادات الحيوية في هذا الوسط.