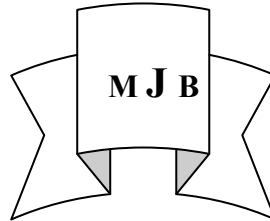


## Effect of human colostrums on *Entamoeba histolytica* in vitro

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### Abstract

This study was done to test the effect of human colostrums on *Entamoeba histolytica* trophozoite in culture media. Concentration of 5%, 10%, 15% and 20% of human colostrums were added to liver infusion agar containing *E. histolytica* trophozoites then incubated at 37°C for (60, 120, 180, 240 and 300) minutes. At the end of incubation periods the number of trophozoites was recorded, the results showed an inhibitory effect of human colostrums on *E. histolytica* trophozoites especially in high concentration which resulted in killing all trophozoites in culture media.

### تأثير لبئ الحليب على الأطوار المتغذية للاميبيا الحالة للنسيج في الزجاج

### الخلاصة

صممت هذه الدراسة لغرض معرفة تأثير لبئ الحليب على الأطوار المتغذية للاميبيا الحالة للنسيج في الوسط الزرعي. أضيفت التراكيز 5%، 10%، 15% و 20% من لبئ الحليب إلى وسط نقيع الكبد الحاوي على الأطوار المتغذية للاميبيا الحالة للنسيج وحضنت عند درجة حرارة 37°م للفترة (60، 120، 180، 240، 300) دقيقة. عند نهاية فترة الحضان تم حساب أعداد الأطوار المتغذية للطفيلي وأظهرت النتائج إن لبئ الحليب فعالا في القضاء على الطفيلي وخصوصا عند استخدامه بالتراكيز العالية (20%).

### Introduction

**E***ntamoeba histolytica* is a pathogenic parasitic protozoan that can infected the large intestine and causes amoebiasis, an illness responsible for at least 100.000 deaths annually worldwide (Boon et al., 2006). When ingested, cyst passes through the stomach and excyst in the distal part of the small intestine and colon. The emerging trophozoites lives in the luman of the bowel close to the mucosa, where they feed by phagocytosis on particulate matter and bacteria and by pinocytosis on liquid

nutrients. They may invade the gut wall and produce ulceration and subsequent dysentery. *E. histolytica* trophozoite may be transported by the blood to extraintestinal locations such as liver, lungs and brain (Haque et al., 2003).

Human colostrums is a widely recognized healthy and nutritious source of energy, proteins, vitamins and minerals (Luthfor et al., 2004). In addition to the nutritional value of colostrums, it play a protective role in its capacity to kill microbes (Ardythe & Josefa, 2004). Colostrums contain immunoglobulins which are protective proteins important in the transfer of passive immunity from the mother to

the neonate ( Hanson & Korotkova, 2002). Also colostrums contains other proteins such as lactoferrin, lactoperoxidase, lysozyme and N-acetyl- $\beta$ - glucosaminidase are through to have antimicrobial activity (Lonnerdal, 2004).

The demonstration of antibodies in breast milk may have epidemiological significance in population studies. Antibodies of IgA class were found in serum and colostrums of parturient women in an endemic area of amebiasis (Berber et al., 1990).

The aim of this study was to investigate the effects of human colostrums in killing *E. histolytica* trophozoite. And preventing infection caused by infected water and food in breast – fed children.

## Materials and Methods

### 1- Culture media:

*E. histolytica* fresh clinical isolate was obtained from a patients with acute amebiasis. Trophozoites were cultured in liver infusion agar for 48 hours at 37°C, at the end of the period amoebae reached logarithmic growth phase (Mirelman et al., 1987). Trophozoites were chilled for 10 minutes in an ice water bath, the vigorously shaken to detach amoebae adherent to the walls of the tube then counted with a haemocytometer and then  $20 \times 10^5$  amoeba/ ml were transferred to fresh medium.

Five tubes contained 5 ml liver infusion agar were prepared for each groups, then colostrum was added in at different concentrations (5%, 10%, 15% and 20%) while the fifth tube was used as a control. Tubes were incubated at 37°C for (60, 120, 180, 240 and 300) minutes respectively. At the end of incubation periods the number of trophozoites were counted by using haemocytometer with trypan blue stain (Mirelman et al., 1987). All

experiments were done in triplicate and repeated at least twice.

### 2- human colostrums collection:

Fresh human colostrums were collected from 5 healthy lactating mothers with no clinical evidence of infection, who voluntarily donated up to 10 ml of colostrums by breast pump. Colostrums were obtained during the first three day of lactation and collected from mothers between 9 and 12 a.m., just before they began to nurse their babies. The colostrums were collected in clean and sterile tubes then kept in refrigerator until use.

### 3- statistical analysis:

Program( SPSS) was used to analyzed data by using F- test. Analysis was performed probability values less than 0.05 considered statistically significant (Niazi, 2004).

## Results and Discussion

The results showed an inhibitory effects of human colostrums on *E. histolytica* trophozoite in culture media. the number of living trophozoites were decreased with increasing concentration of colostrums. The results showed that the best lethal effect (50% killing) was obtained from all concentration during the first 60 min of added colostrums. It was also observed that there are no living *E. histolytica* trophozoites at the end of 300 minute incubation when used 20% human colostrums (figure 1).

A significant lethal effect of different concentrations of colostrums on *E. histolytica* was observed during 60 min and 180 min exposure ( $p < 0.05$ ). The results are in agreement with Hanson. Korotkova (2002) and Al-Masoudi (2006) who reported that breastfeeding significantly protects children from diarrhea infection. Colostrums may play a protective role

in breastfeeding children exposure to some intestinal parasite such as *Giardia lamblia* due to its contains of immunoglobulins, mainly secretory immunoglobulin A (sIgA) that may be play a role in such protection (Korhonen et al, 2000). Grundy et al.(1983) demonstrated sIgA antibodies to *E. histolytica* in milk (31%) and serum (14%) of mothers living in endemic areas, anti-Eh sIgA

antibody titers were significantly increased in colostrums samples of mothers of newborn children with diarrhea (Leon- Sicairos et al., 2006).

In this study, we showed that colostrums has direct lethal effect on *E. histolytica* in vitro. Therefore, when trophozoites that emerge from cysts in the small intestine are exposed to colostrums, they may be killed.

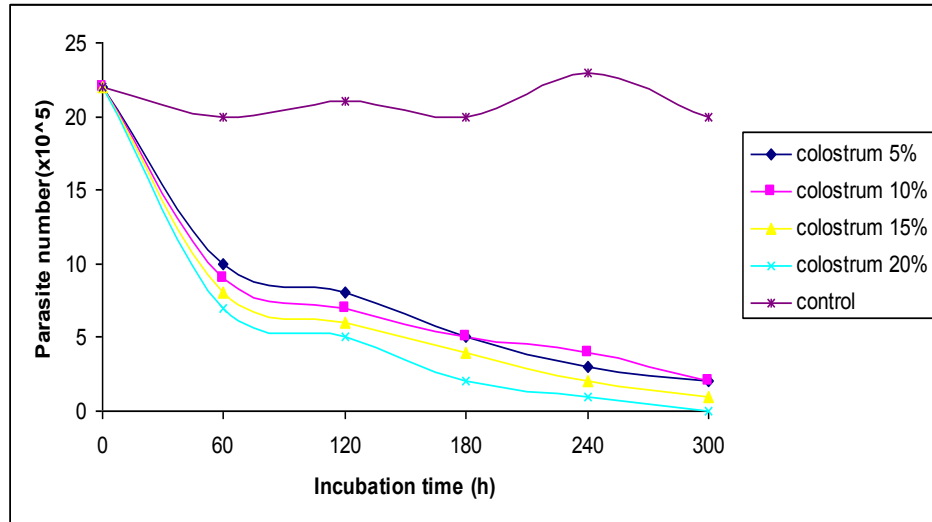


Figure 1: Effect of human colostrums on *E. histolytica* trophozoite

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