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# Effect of Lactobacillus Acidophilus on IL-17 Expression Gene Levels in the Liver of Fetal Mice Infected with Entamoeba Histolytica

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**Annotation:** Background & aim: Gram-positive lactic acid-producing bacteria belong to the broad and varied family Lactobacillaceae. Many mammals have gut microbiota, which includes these healthy bacteria that are commonly utilized as probiotics. Therefore, the study aimed to evaluate the effect of Lactobacillus Acidophilus on IL-17 expression gene levels in the liver of fetal mice infected with Entamoeba histolytica.

**Materials & methods:** 40 distinct traditional yoghurts were obtained from Kirkuk marketplaces in the area at March 2025. while, 85 Random stool samples were taken from diarrheal patients at Azadi Teaching Hospital between March to Aril 2025. 20 adult albino mice were acquired from Tikrit University's Veterinary College and mice were divided as follow (4 female and male in each group). Control group. Female mice injected E. histolytica. Female mice treated with Lactobacillus acidophilus. Female mice injected with E. histolytica and treated with Lactobacillus acidophilus concentrated filtrate for two weeks. The fetal liver was carefully cut up and spread out to small sections for histological and gene expression study.

**Results:** The current study's findings

indicate that the overall prevalence of E. histolytica was 15/85 (17.6%). for histological examination, fetal livers of the infected group showed thickening of the central vein wall with degeneration and necrosis of hepatocytes, and decreased in hematopoietic elements, while, in L. acidophilus group, Sections prepared from the livers of fetuses in this group were similar to the normal group, as the central veins and hepatocytes were normal. in treated group, Cross-sections of the livers of fetuses in this group showed improvement compared to the infected group, as there was an improvement in hepatic cells, the amount of hematopoietic elements, and megakaryotic cells, but there continued to be thickening of the walls of some blood vessels and degeneration of some hepatic cells. The results showed that gene expression in studied groups, the mean of folding changes in infected group was 6.592, while in control, folding changes was 8.267. In L. acidophilus group, folding changes was 13.098. In treated group, folding changes ranged was 9.062. The results showed a significant (P=0.001) increase in gene expression in patients compared to the control group.

**Conclusions:** it was found that infection with E. histolytica led to an increase in the expression levels of the IL-17 gene in the liver of fetal mice, while Lactobacillus Acidophilus played an effective role in regulating the expression of the IL-17 gene and liver protection.

**Keywords:** E. histolytica, IL-17, Lactobacillus, Liver, Expression gene.

## Introduction

Numerous "probiotic" health products have taken over the global market since the early 1990s. Meanwhile, "probiotics" have emerged as a popular worldwide study area. Probiotics have been shown to produce a range of potential health effects and have been thoroughly studied in a variety

of diseases. Yeasts, bifidobacteria, and lactobacilli are the most researched species [1,2]. Due to its direct connection to human health, L. acidophilus, a significant intestine probiotic in the lactic acid bacteria (LAB) family, has received an abundance of interest in terms of study and development. As a result, L. acidophilus is one of the most often suggested bacteria for dietary usage and is generally thought to provide probiotic properties [3,4]. Gram-positive Lactobacillus acidophilus is a member of the Lactobacillus genus in the Lactobacillaceae family [5]. It has been demonstrated that L. acidophilus, a species of beneficial microbial flora, possesses numerous positive probiotic traits. Previous studies have shown that L. acidophilus plays a role in controlling the body's capacity to react to immune responses. In immunodeficient mice, NCFM elicited antibody- and cell-mediated responses to Candida albicans, as Wagner et al. confirmed [6,7]. Otherwise, Entamoebahistolytica is an intestinal parasite that causes amebiasis. According to multiple studies, it infects 50 million people worldwide and kills between 40,000 and 100,000 people each year, making it one of the leading causes of death from parasitic infections like schistosomiasis and malaria [8,9,10]. Most tropical and subtropical nations have endemic E. histolytica [11. Both intestinal and extraintestinal amebiasis infections are possible, particularly in the liver; intestinal amebiasis can range greatly in severity from no symptoms to dysentery. E. histolytica cysts in water or food tainted with excrement are the most prevalent way for the host to become infected with amebiasis [11,12]. Therefore, the study aimed to evaluate the effect of Lactobacillus Acidophilus on IL-17 expression gene levels in the liver of fetal mice infected with *Entamoeba histolytica*.

## Materials & methods

### 1. L. acidophilus

40 distinct traditional yoghurts were obtained from Kirkuk marketplaces in the area at March 2025. Upon delivery at the laboratory, all samples were refrigerated and kept at 4°C until they were analyzed.

### 1.1. Isolation of Bacteria

The dilution agar method was used to isolate many bacterial strains from these food sources. Plated on de Man, Rogosa and Sharpe (MRS) (HIMEDIA, India), 0.1 ml of the yoghurt dilutions (with normal saline) were incubated for 48 hours at 37°C in an anaerobic environment. Gram stain and catalase production were used to examine the pure colonies under a microscope for lactobacilli. For additional research, the gram-positive, catalase-negative rods were chosen. MRS agar media and modified MRS broth were used to isolate the bacteria Lactobacillus spp. from yoghurt samples. The isolation procedure was finished using the Issazadeh et al. approach. [13]

### Stool and Parasite diagnosis

85 Random stool samples were taken from diarrheal patients at Azadi Teaching Hospital between March to Aril 2025. For confident the parasite was present, a small sample was examined using a direct microscope. Cysts of Entamoeba histolytica were isolated in accordance with Khairnar and Parija [14] from the feces of an infected patient.

### Animal Model

20 adult female albino mice (weight 20–25 gm, ages 3-5 months) were acquired from Tikrit University's Veterinary College and fed a typical pellet diet for two weeks to make sure everything was normal and free of infections.

### Experimental design

The parasite infection was carried out on the first day of pregnancy, and treatment with Lactobacillus acidophilus concentrated filtrate was carried out after confirming pregnancy and infection on the fourth day. Treatment continued until the 18th day of pregnancy.

20 adult albino mice were used in this study and then divided as follow (4 female and male in each group)

- Control group received standard pellet diet only.
- Female mice injected (intraperitoneal) with E. histolytica at dose $10^3$ cyst/ml [15].
- Female mice treated with Lactobacillus acidophilus concentrated filtrate (500 mg/kg) (orally) [16] for two weeks.
- Female mice injected (intraperitoneal) with E. histolytica and treated with Lactobacillus acidophilus concentrated filtrate for two weeks.

**Histology samples**

The fetal liver was carefully cut up and spread out to small sections before being immersed in formal saline (10%). Gradual ethanol (HiMedia, India) was used to dehydrate the liver pieces, cleared with xylene (HiMedia, India), wax was embedded, sectioned at 7um, stained with hematoxylin and eosin (HiMedia, India), and then examined under a microscope [17,18].

**RNA Isolation**

The TRIzol Reagent was used to homogenize the fetal liver in order to extract the RNA. The extract was then centrifuged using the Transzol Up Plus RNA kit (Cat. No. ER501) at 14000 ×g in a column tube in accordance with the manufacturer's instructions. chloroform extraction and ethanol precipitation were used to obtain and purify the RNA pellet, which was then further dissolved in TAE buffer pH 8.0. Real-Time PCR (qRT - PCR) One-step qRT-PCR kit (TransZol Up Plus, China) was used to study the mRNA expression. Primers were designed using forward and reverse sequences, as shown in Table 1.

Table 1. Primers designed for qPCR

| Primer | Sequences | Ref. |
|---|---|---|
| IL-17 F | GAG CTT CAT CTG TGT CTC TG | [19] |
| IL-17 R | GAG GTT GAC CTT CAC ATT CT | |
| GAPDH-F | TCA AGA TGG TGA AGC AG | |
| GAPDH-R | ATG TAG GCC ATG AGG TCC AC | |

The qPCR reaction was preceded by 5 minutes of reverse transcription at 42 oC and 3 minutes of reverse transcriptase (RT) enzyme inactivation at 95 oC. For the PCR to go through its various stages, temperature cycling is necessary. The primer was annealed at 60 oC for 20 s, the dsDNA was denaturated for 30 s at 95 oC, and the extension was performed for 20 s at 72 oC. The Ct value was used to present the findings. For both the internal control gene (GAPDH) and the targeted genes, the average Ct value was computed. The ΔCt was calculated by comparing the threshold cycles of GAPDH and the targeted genes. The 2-ΔΔCt was used to determine gene expression [20].

**Statistical analysis**

The differences between two groups and between multiple groups were assessed using one-way analysis of variance (ANOVA), respectively. Mean ± standard deviation (SD) was used to report the results. GraphPad Prism 7.0 (USA) was used for statistical analysis, and a difference is deemed statistically significant if it is less than 0.05.

**Results & Discussion**

**Sample distribution**

An examination was conducted on 85 individuals who experienced watery diarrhea and abdominal pain. Fecal samples were found using microscopy. The current study's findings indicate that the overall prevalence of E. histolytica was 15/85 (17.6%) (Table 1). These findings concur with those of Ali et al. [21], who found that the frequency of E. histolytica in Bangladeshi preschoolers was 15.6%. In seven Malaysian villages, Aza et al. [22] found that intestinal parasite prevalence was 21.0%. Children in Delhi, India, and Pakistan, respectively, had an 11% prevalence of E. histolytica, according to studies by Zahida et al. [23].

**Table 2: *Entamoeba histolytica* microscopically positive samples**

| Procedures | Samples | Positive samples | |
|---|---|---|---|
| | | No. | % |
| Direct examined (wet mount) | 85 | 15 | 17.6% |

### Histological study

After confirming that pregnant mice were infected with the parasite using microscopic examination to detect the cystic stage on the fourth day of pregnancy, the groups were divided into four according to the type of treatment. The mice were dissected on the 18th day, and the livers of the fetuses were extracted and examined under the microscope.

### Control group

The livers of the control group fetuses showed normal hepatocyte morphology with the presence of hematopoietic elements (HPE), red blood cells, and normal sinusoids in the lobules of the liver sections (Figure 1).

### Parasite infected group

Livers of the infected group showed thickening of the central vein wall with degeneration and necrosis of hepatocytes, hematopoietic elements, and red blood cells were few compared to the control group, and there was necrotic material in the sinusoids (Figure 2).

### *L. acidophilus* group

Sections prepared from the livers of fetuses in this group were similar to the normal group, as the central veins and hepatocytes were normal and their arrangement was normal, and the hematopoietic elements were normal and distributed normally in the sinusoids (Figure 3).

### Treated group

Cross-sections of the livers of fetuses in this group showed improvement compared to the infected group, as there was an improvement in hepatic cells, the amount of hematopoietic elements, and megakaryotic cells, but there continued to be thickening of the walls of some blood vessels and degeneration of some hepatic cells.

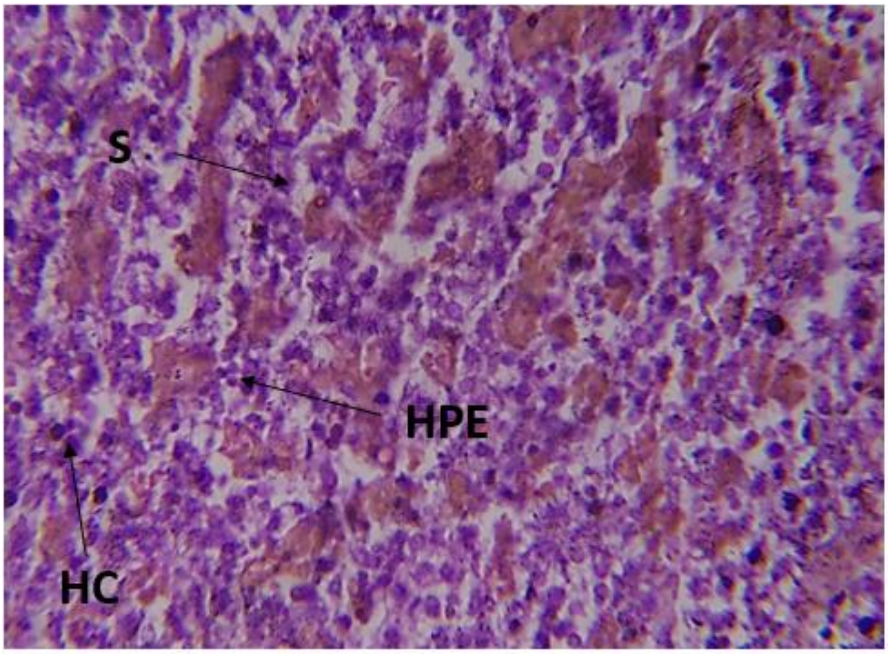


Figure 1: Liver of a control fetus showed hepatocytes (HC), sinusoids (S) with the presence of hematopoietic elements (HPE), and red blood cells H&E X400.

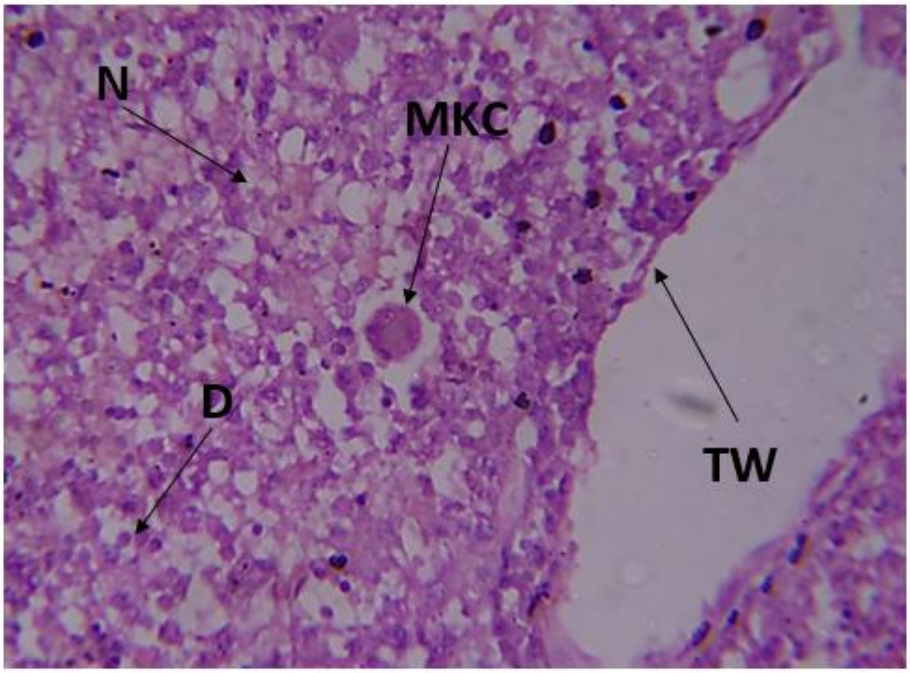


Figure 2: fetus liver of infected group showed thickening of the central vein wall (TW) with degeneration (D) and necrosis (N) of some hepatocytes, Megakaryocytes (MKC) H&E X400.

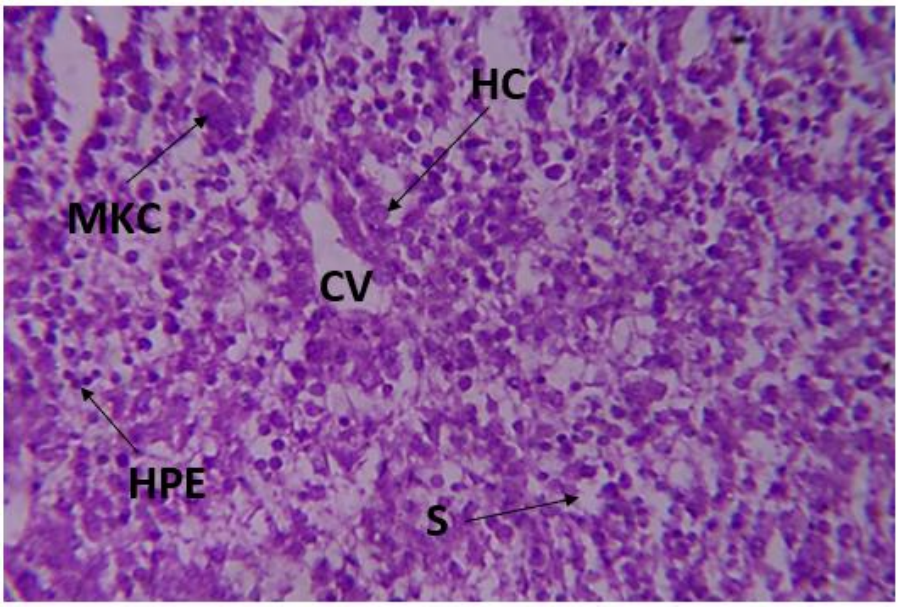


Figure 3: fetus liver of L. acidophilus group showed normal central vein (CV), hepatocytes (HC), sinusoids (S) with the presence of hematopoietic elements (HPE), and megakaryocytes (MKC) H&E X400.

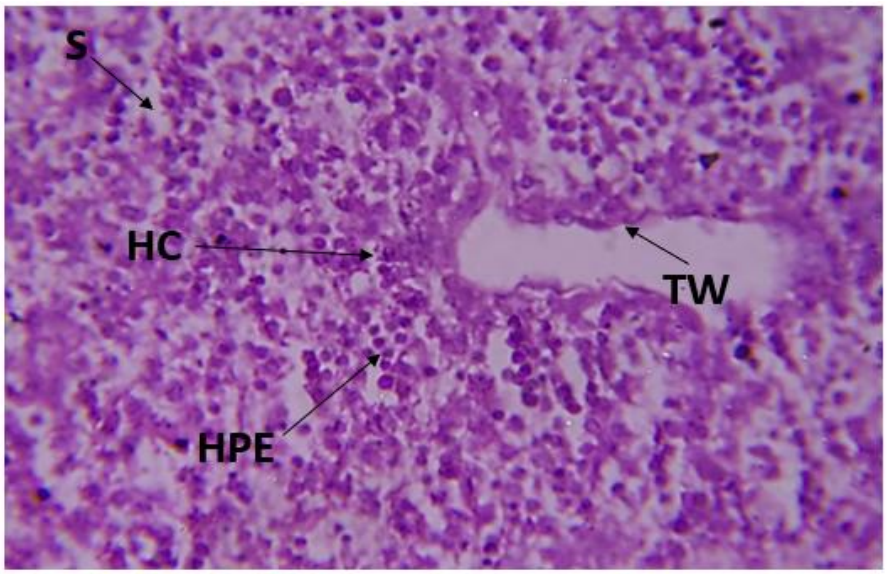


Figure 4: fetus liver of infected and $L_g$ acidophilus group showed thickening central vein wall (TW), hepatocytes (HC), sinusoids (S) with the presence of hematopoietic elements (HPE) H&E X400.

After intraportal inoculation with *E. histolytica*, an amoebic liver abscess forms in three stages: acute inflammation, abscess development, and necrosis. A week following infection, tissue necrosis results from microabscess development and hepatocyte injury, indicating that amoebic particle diffusion occurs in the endothelium and that distant hepatocytes perish from necrosis [24,25]. Even in the absence of direct trophozoite-hepatocyte interaction, these authors hypothesize that cytotoxicity may be caused by the production of amoebic particles, which have the capacity to manifest detrimental effects remotely. This study discovered that the longer the incubation period, the more apoptotic cells there were. One crucial aspect of apoptosis was the presence of broken nuclei. A study Carranza-Rosales et al. [26] found that the growth of amoebic liver abscess (ALA) results in significant loss of liver tissue, indicating a gradual increase in apoptosis in infected slices as the incubation period increases. These findings were consistent with those previously described, and probiotics shown a noteworthy capacity to preserve the cellular integrity of the liver [27]. Pregnant mice in the probiotic-treated group showed fewer histological alterations in their feteus, indicating Lactobacillus acidophilus's beneficial effect on liver function. Probiotics' positive effects on the liver suggested that they are well tolerated, can enhance liver function, and may lower the lipid peroxidation marker [28]. According to the role of probiotics in liver illness, the primary advantages of probiotics for liver health may be found in their ability to inhibit the gut's synthesis and/or absorption of lipopolysaccharides, which in turn lowers low-grade inflammation [29]. The beneficial effects of probiotic bacteria as an adjuvant may be explained by the active metabolite released by these bacteria during intestinal transit, which may cross the intestinal layer and exert anti-inflammatory effects [11]. This is explaining the role of L. acidophilus in improving the liver of mice in the treated group after infection in the current study.

**IL-17 gene expression**

Table (3) shows that gene expression in studied groups, the mean of folding changes in infected group was ranged (2.867-19.973) with an average of 6.592, while in control, folding changes ranged from (5.278-11.313) with an average of 8.267. in L. acidophilus group, folding changes ranged from (7.061-19.973) with an average of 13.098. in treated group, folding changes ranged from (6.147-13.177) with an average of 9.062. The results showed a significant (P=0.001) increase in gene expression in patients compared to the control group. In Figure (5) Curves of amplification were the X-axes represent the number of cycles while Y-axes represent in testing Florence. Positive samples showed curve shape of amplification at different cycle number on Roy channel, while negative specimens display know curve amplification at any point of the cycle number and will remain flit blow the threshold level of amplification.

Table (1): The expression level of IL-17 gene in fetal liver groups.

| Groups | IL-17 | GAPDH | ΔCT | ΔΔCT | 2-ΔΔCt | Folding | Mean | P value |
|---|---|---|---|---|---|---|---|---|
| Control group | 27.1 | 21.9 | 5.2 | -3.1 | 8.5741877 | 8.5741877 | 8.267995 | 0.001 |
| | 28.7 | 22.8 | 5.9 | -2.4 | 5.278031643 | 5.278031643 | | |
| | 26.6 | 21.8 | 4.8 | -3.5 | 11.3137085 | 11.3137085 | | |
| | 27.4 | 21.7 | 5.7 | -2.6 | 6.062866266 | 6.062866266 | | |
| | 26.9 | 21.8 | 5.1 | -3.2 | 9.18958684 | 9.18958684 | | |
| Infected group | 26.8 | 21.4 | 5.4 | -1.52 | 2.867910496 | 2.867910496 | 6.592148 | |
| | 27.4 | 22.2 | 5.2 | -1.72 | 3.294364069 | 3.294364069 | | |
| | 26.1 | 20.9 | 5.2 | -1.72 | 3.294364069 | 3.294364069 | | |
| | 25.3 | 22.7 | 2.6 | -4.32 | 19.97328878 | 19.97328878 | | |
| | 26.9 | 21.8 | 5.1 | -1.82 | 3.530811985 | 3.530811985 | | |
| L. acidophilus group | 25.7 | 22.6 | 3.1 | -3.82 | 14.12324794 | 14.12324794 | 13.09864 | |
| | 26 | 22.1 | 3.9 | -3.02 | 8.111675838 | 8.111675838 | | |
| | 25.1 | 22.2 | 2.9 | -4.02 | 16.22335168 | 16.22335168 | | |
| | 26.3 | 22.2 | 4.1 | -2.82 | 7.06162397 | 7.06162397 | | |
| | 25.5 | 22.9 | 2.6 | -4.32 | 19.97328878 | 19.97328878 | | |
| Treated group | 26.3 | 22.1 | 4.2 | -2.72 | 6.588728138 | 6.588728138 | 9.062197 | |
| | 25.5 | 22.3 | 3.2 | -3.72 | 13.17745628 | 13.17745628 | | |
| | 25.9 | 22.1 | 3.8 | -3.12 | 8.6938789 | 8.6938789 | | |
| | 26.8 | 22.5 | 4.3 | -2.62 | 6.147500725 | 6.147500725 | | |
| | 25.8 | 22.3 | 3.5 | -3.42 | 10.70342044 | 10.70342044 | | |

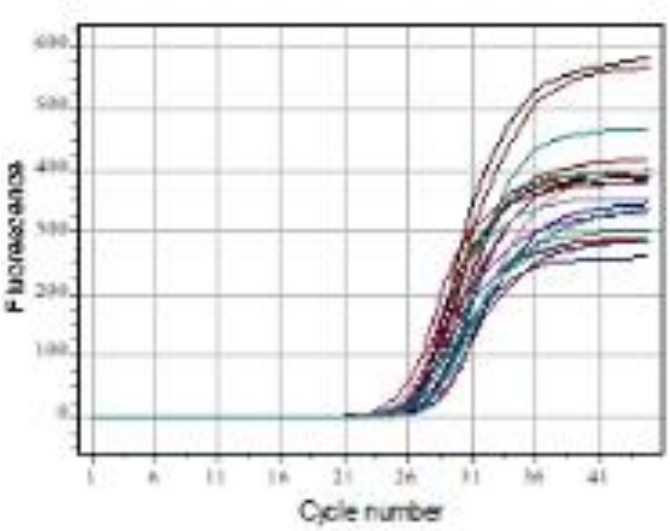


Figure (5): IL-17 gene amplification on the Cy5 channel shows a positive sample with a curve shape at various cycle numbers.

According to earlier research, mice who are given a large dose of parasitic cysts orally experience a strong inflammatory reaction [31,32]. Tissues from mice at day 7 p.i. were subjected to histological investigation as previously described [33] to ascertain whether a lack of IL-17 can change the inflammatory response. Hepatocytes in the liver of mice infected with fetul exhibited degenerative alteration, as was to be expected. This supports the theory that decreased IL-17 receptors and, as a result, downregulated IL-17 gene expression caused liver damage and histological alterations in the liver of infected fetuses. With purported probiotic benefits, Lactobacillus acidophilus is one of the most common commercial species of lactic acid bacteria found in various dairy products and dietary supplements [34]. Studies have shown that Lactobacillus can improve the expression of certain genes in the liver and epithelial cells. For example, Zhao et al. [35] found that in a mouse model of

chronic ethanol exposure, Lactobacillus rhamnosus protected the liver from ethanol-induced liver inflammation by lowering ethanol-elevated miR-122a . Kalani et al. discovered that L. acidophilus suppressed the pro-inflammatory molecule miR-155 in human umbilical vein endothelial cells treated with LPS [36]. The treatment of L. acidophilus 1.0738 to sensitized mice was reported to significantly decrease the production of IL-17, TNF-α, and IL-6, as well as to increase TLR2 transcription and protein levels in colon tissue [37].

## Conclusions

Based on the results of the current study, it was found that infection with E. histolytica led to an increase in the expression levels of the IL-17 gene in the liver of fetal mice, while Lactobacillus acidophilus played an effective role in regulating the expression of the IL-17 gene. This proves the effectiveness of bacteria in improving the tissues of fetal liver from parasitic toxins.

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