Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 13s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

A Diagnostic Study Of Amoebic Dysentery And Its Effect On Some Immune Indicators In Children

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Cite this paper as: Dunia Abed Hussein Al-Tikrity, Hanan Adhawi Abdullah, Khansaa Ahmed Hasan, (2025) A Diagnostic Study Of Amoebic Dysentery And Its Effect On Some Immune Indicators In Children. *Journal of Neonatal Surgery*, 14 (13s), 801-806.

ABSTRACT

This research was conducted on children infected with the *Entamoeba histolytica* parasite who arrived at Tikrit Teaching Hospital in Salah al-Din Governorate in the month of June 2024 to December 2024 on children aged 1-6 years and for both sexes. Stool samples were collected for children with acute diarrhea. 100 faecal samples were examined and 20 positive samples were recorded using the direct swab method. The results of the research showed a total infection rate with the *E. histolytica* parasite that reached 20%, and the infection rate in females reached 28%, higher than that in males, reaching 12%. The highest percentage was found at the age of 5-6 years, reaching 25%, and the lowest rate was in the age group 1-2 years, reaching 10%.

The highest rate of dysentery among children was in Baiji, 36%, and the lowest rate was recorded in Tikrit, 8%.

The highest infection rate was in July, at 53.3%, while the lowest infection rate was in September, at 0%. The effect of dysentery on some immune parameters in the blood, such as Immunoglobulin A and Interferon gamma, was studied. There was a high increase in the concentration of interferon gamma in children infected with *E. histolytica*, reaching 1512 compared to healthy people, reaching 834, as well as an increase in the concentration of IgA in infected children, reaching 5.83 compared to healthy people, reaching 3.68.

1. INTRODUCTION

Entamoeba histolytica is a protozoan that infects the intestine and causes amoebic dysentery (1) By consuming food and water contaminated with the cyst stage (2) The common hosts of this parasite are humans, dogs, monkeys, pigs, cats, rats, and mice. The infection poses a major threat to global health because it spreads throughout the world (3). This parasite settles in the large intestine and causes symptomatic amoebiasis to the host, and it is called the carrier. The parasite causes damage to the cells, causing painful cup ulcers and thus causing amoebic dysentery (4). Infection with the parasite stimulates the host's natural immune response, as mucous substances and lytic enzymes are secreted by the mucous membranes lining the digestive system and bowel movements, and they represent natural defensive means that the body displays to eliminate the parasite (5). The humoral immune response is also stimulated, as an increase in the level of IgG, IgM, and IgA antibodies was observed. Interferon is one of the cytokines that are affected when infected with this parasite, as it plays an important role in controlling the parasite, as it stimulates immune cells, especially neutrophil cells, which have an important role in attacking the parasite (6).

2. MATERIALS AND METHODS

Collect stool samples: 100 stool samples were collected from children arriving to Tikrit Teaching Hospital in Salah al-Din Governorate The period for collecting samples was from the beginning of May 2024 until the end of December 2024, and included samples from males and females, with ages ranging between (1-6) years. Stool samples were stored in sterile, dry plastic bottles equipped with a tight stopper, and the patients' information was recorded (gender, age, residence). The samples were examined with the naked eye to determine the consistency, color, and odor of each stool sample.

Collecting blood samples:

3 milliliters of blood were drawn from each child suffering from diarrhea and diagnosed with amoebic dysentery, and the same volume of blood was taken from unaffected children as a control.The blood sample was placed in a fine test tube that did not contain an anticoagulant. It was left for 15 minutes at 37 degrees Celsius, then centrifuged for 5 minutes at a speed of 3000 revolutions per minute. The serum was then placed in clean tubes, and the samples were numbered and then stored at 20 degrees Celsius until immunological tests were performed (7).

Examination stool sample

A stool sample was taken from different parts of the sample using a wooden instrument similar to a stick.

After that, the stool sample was mixed with physiological salt on a clean glass slide, then we put the cover of the glass slide and examined with a microscope with a 100 x oil lens to confirm the presence of the parasite (8).

Immunological tests

1- Estimation of IgA immunoglobulin concentration IgA concentration was estimated using the Genrui Kit by the following steps:

Components of the test kit	
1	**Buffer solution**
2	**Antiserum**
3	**Sample Diluent (optional)**
4	**Stirrer**

Action steps:

1- Place all reagents at room temperature (about 25 degrees Celsius) before use.

2- After turning on the device, the main measurement interface was displayed and the test item and sample type were selected in the item column

3- Take a laboratory cuvette, put one stirrer in it, then put 400 microliters using a pipette of buffer solution, then add 4 microliters of the serum sample to it.

4- Place the cuvette in the test channel and turn the device automatically only once.

5- Add Antiserum using a pipette, carefully adding 100 microliters.

6- When the test is finished, the device will automatically display and print the results.

Estimation of interferon gamma INF Y concentration: The INF Y concentration was measured according to the test kit and the steps in sequence.

Action steps:

The ELISA kit contains a smaller plate that was coated previously with the specific INF Y antibodies introduced by the company, then additional samples and scale were put into small holes of the ELISA plate. Then with the designated antibodies, we added the antibody for Avidin peroxidase complex reagent (HRP) designated INF Y to each hole, and after incubation and washing we added later solution TMP to plate's holes.

When they are added the reaction termination solution, the pits containing INF Y and avidin peroxidase HRP coupled to the antibodies appear blue and then appear yellow. Spectrophotometric measurement of optical density is made at 450 nm. The INF Y concentration is proportional to the OD value. The OD in the standard curve is compared to calculate the INF Y concentration in the samples.

Results:

Percentage of infection with the *E.histolytica* parasite in stool samples examined with a microscope Stool samples were collected from children arriving to Tikrit Teaching Hospital, aged from 1-5 years. Samples were collected from June 2024 to December 2024. Microscopic examination of stool of 100 samples showed the parasite in 20 samples and the percentage of 20% (table no.(1-1)).

table (1-1): total parasite infection rate.

total number of samples tested	Number of infected samples	Percentage
100	20	20**%**

The effect of gender on parasite infection:

The incidence of infection in females was 28% and less in males at 12%, as shown in table (2-4).

table (2-4): Percentage of infection with the *E.histolytica* parasite by gender.

Gender of the infected person	Examined people	Infected people	Percentage
Females	50	14	28%
Males	50	6	12%
Total	100	20	20%

The effect of age on the incidence rate:

The highest infection rate was in the age group (5-6) years and amounted to 25%, and the lowest infection rate was recorded in the age group (1-2) years and amounted to 10%, as in table (3-4).

Table (3-4): Percentage of people infected with the *E. histolytica* parasite according to age groups.

Age group	Number of people examined	Number of people infected	Percentage
1-2) years**(**	30	3	10%
3-4) years**(**	30	7	23.3%
(5-6) years	40	10	25%
Total	100	20	100

Infection rate according to area of residence:

The results showed the highest infection rate of 36% in the Baiji area, which is considered one of the areas surrounding the city center, and the lowest infection rate in the Tikrit area, the city center, and reached 8%, as in table (4-4).

table (4-4): Percentage of people infected with the *E. histolytica* parasite, according to areas of residence.

Residential area	Number of children examined	Number of children infected	Percentage
Tikrit	**25**	**2**	**8%**
Al-Alam	**25**	**4**	**16%**
Baiji	**25**	**9**	**36%**
Dor	**25**	**5**	**20%**
Total	**100**	**20**	**20%**

Infection rate by month of the year:

The highest infection rate was in the month of July, 53.3%, and there was no infection in the month of September, 0%, as in table (5-4) Percentage of parasite infection according to the months of the year.

table (5-4) Percentage of parasite infection according to the months of the year.

. Number of months in the year	Examined people	Infected people	Percentage
June	15	1	6.6
July	15	8	53.3
August	15	2	13.3
September	15	0	0
October	15	1	6.6
November	15	5	33.3
December	10	3	30
Total	100	20	100

Measuring the rate of immune indicators:

The research results showed a high percentage of INFY for children

Those infected with aponeurosis and children who were not infected with the control samples. Also, as for the IgA type, its quantity was found to be higher when compared with the control samples, as shown in Table (6-4).

Table (6-4): The rate of immune indicators for those infected compared to healthy people.

Group	INF_Y	IgA
Patients	1512	5.83
Control	834	3.68

3. DISCUSSION

The infection rate in the research was consistent with (9) in Tikrit, and a higher rate was recorded in females than in males, as it was 27.18% in females and 25.19 in males. It was also consistent with what (10) found in Tikrit as well, as the infection rate in females reached 11% while in males it was 9%, and it differed with what (11) found. In Kirkuk, where a higher infection rate was recorded in males than in females, reaching 31.9% in males and 26.5% in females. This difference may be due to environmental and physiological factors and exposure to the pathogen as a result of the difference between the behavior of males and females (12). The highest infection rate was 25% at the age of (5-6) years, and the lowest rate was 10% at the age of (1-2) years. This percentage was identical to (11) in Kirkuk, where the highest infection rate was recorded in children in the age group of 5-6 years. The highest infection rate was in the Baiji area, 36%, and the lowest in Tikrit, which was 8%, and I agreed with (13) in Samarra, as the highest infection rate was obtained in village areas as a result of the lack of services and the high degree of pollution, which makes them more vulnerable to parasitic diseases than others due to poor health services, in addition to the lack of safe drinking water and the lack of good sanitation of water (14). The highest rate of dysentery infection was in July, 53.3%, while there was no infection in September, and I agreed with (11), which recorded a rate of 44.3% in July, and a lower rate of 5.8% in January. This increase is due to the appropriate humidity and temperature for the presence of the cystic stage of the parasite that causes infection and its persistence for a long period (15).

The results of an increase in the INFy immune index for infected children agreed with (16) in Anbar Governorate, as a high significant increase in this index was recorded and the close relationship between INFy and infection with the parasite was proven, as INFy works to stop the production of proteins for the parasite by binding to special receptors in the parasite's membrane that end up stopping the construction of the parasite's own proteins (17). INFy also has a role in reducing infection by activating phagocytic cells that kill Parasite (18)

The results also showed an increase in IgA antibodies in people with dysentery compared to those without dysentery, and they agreed with (19) in Kirkuk Governorate, as the increase in IgA production causes the cells lining the intestines to produce these antibodies, which depends on the presence

of microorganisms in the intestines (20).

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