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Laboratory and Statistical Analysis of Children's Infection with the Amoeba Parasite and its Impact on Immune Indicators in Salah Al-Din Governorate

Dunia Abed Hussein Al-Tikrity
Department of Life Science, College of Education for Women, Tikrit University, Iraq,
dunia_abed@tu.edu.iq

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Abstract: This study was conducted on children aged 1 to 6 years of age, both male and female, infected with the parasite Entamoeba histolytica.

These children were admitted to Tikrit Teaching Hospital in Salah al-Din Governorate between June and December 2024. Stool samples were collected from children with acute diarrhea. One hundred samples were tested, and twenty tested positives using the direct swab method. The results showed an overall infection rate of 20%, with a higher rate among females (28%) compared to males (12%). The highest infection rate was recorded among children aged 5-6 years (25%), while the lowest was in the 1–2-year age group (10%).

At the regional level, the highest infection rate among children with bloody diarrhea was in Baiji (36%), while the lowest was in Tikrit (8%). In terms of the monthly distribution of infection, July recorded the highest infection rate at 53.3%, while September recorded the lowest at 0%.

The research investigated the effect of

bloody diarrhea on certain immunological markers in the blood, such as Immunoglobulin A and Interferon gamma. A significant increase in Interferon gamma concentration was observed in children infected with E. histolytica, reaching 1512 compared to healthy children whose concentration was 834. IgA concentration also increased in infected children, reaching 5.83 compared to 3.68 in healthy children.

Keywords: Diarrhea in children - Parasitic infection - Bloody diarrhea

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) see figure1.

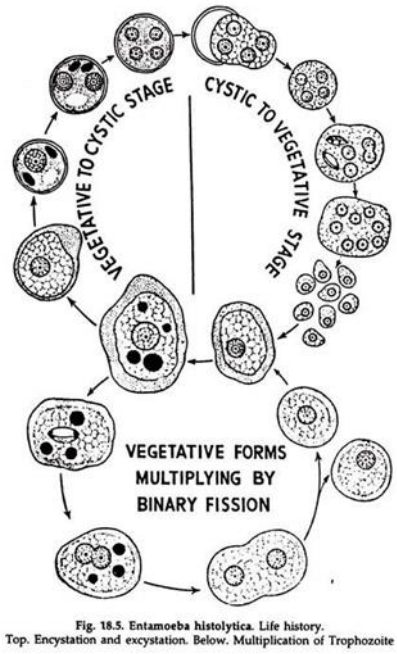

Fig. 18.5. Entamoeba histolytica. Life history.
Top. Encystation and excystation. Below. Multiplication of Trophozoite

Figure 1: Common hosts and mode of parasite transmission

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). see figure 2

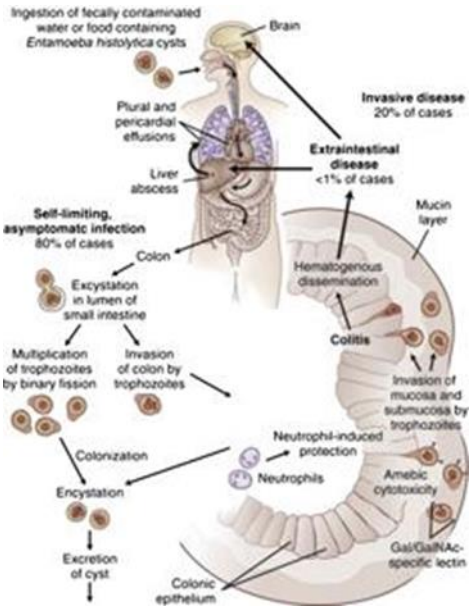

Figure2: Different hosts and the transmission cycle of Entamoeba histolytica through the environment

Materials and Methods

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. See figure3

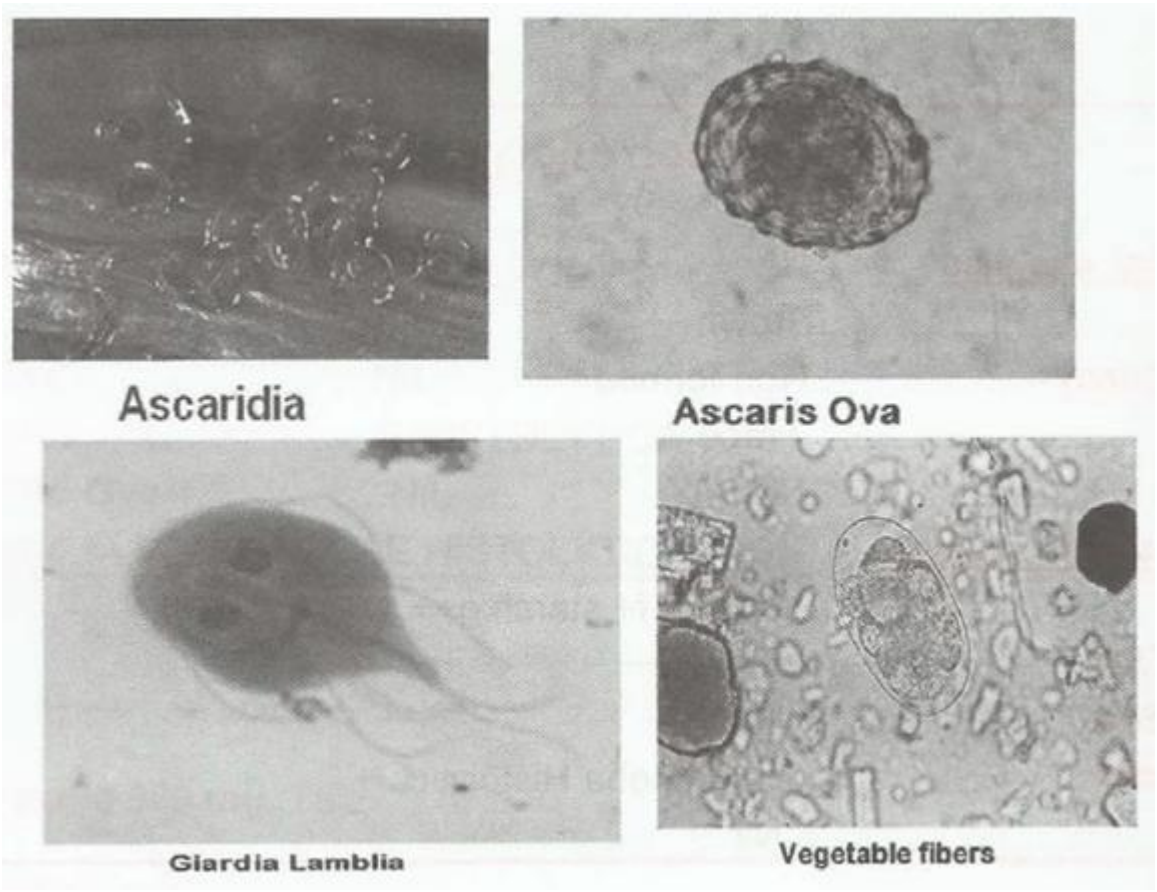

Figure (3) The examined sample of stool and blood samples in the study

Three 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). 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). Estimation of IgA immunoglobulin concentration was done using the Genrui Kit by following the steps which include placing all reagents at room temperature (about 25 degrees Celsius) before use, after turning on the device, the main measurement interface was displayed and the test item and sample type were selected, then a laboratory cuvette was taken, one stirrer put in it, then 400 microliters of buffer solution added using a pipette, then 4 microliters of the serum sample was added. The cuvette was placed in the test channel and the device turned automatically only once. Antiserum was added using a pipette, carefully adding 100 microliters. When the test finished, the device automatically displayed and printed the results. The concentration of interferon gamma INF Y was measured according to the test kit steps. The ELISA kit sandwich system consists of a small plate previously coated with specific INF Y antibodies from the company, samples and scale were added to small holes in the ELISA plate with designated antibodies, then antibody for the avidin peroxidase complex reagent - HRP designated INF Y was added to each hole, followed by incubation and washing, then TMP solution was added to the holes. Pits containing INF Y and avidin peroxidase HRP coupled to the antibodies appeared blue in color and then turned yellow after reaction termination solution was added. Optical density was measured spectrophotometrically at 450 nm. The OD value is

proportional to the INF Y concentration. Concentration was calculated by comparing the OD in the standard curve.

Result:

Samples of stool were collected from children aged 1 to 5 years who visited Tikrit Teaching Hospital between June and December 2024. Microscopic examination revealed that 20% of the samples (20 out of 100) were positive for the parasite E. histolytica.

Infection rates differed by gender, with females showing a higher prevalence (28%) compared to males (12%). Age also influenced infection rates, as the highest percentage (25%) was observed in children aged 5 to 6 years, while the lowest (10%) was found in the 1 to 2 years age group.

Geographically, the highest infection rate occurred in the Baiji area at 36%, whereas the lowest was in Tikrit city center at 8%. Month-by-month analysis revealed that July had the highest infection rate of 53.3%, with no infections detected in September.

Immune indicators measured showed elevated levels of INFY and IgA in infected children compared to healthy controls, suggesting an immune response associated with infection.

Table (1) shows all infection rates data by sex, age, region, and month, based on the information mentioned in the paragraph:

Table (1) Infection Rates Data

Classification	Category	Number Examined	Number Infected	Infection Rate (%)
Gender	Female	50	14	28
	Male	50	6	12
Age	1-2 years	30	3	10
	3-4 years	30	7	23.3
	5-6 years	40	10	25
Area	Tikrit	25	2	8
	Al-Alam	25	4	16
	Baiji	25	9	36
	Dor	25	5	20
Month	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

Table (2) shows the immunity indicators that compare infected and healthy children:

Table (2) immunity indicators

Indicator	Group	Mean Value
INFY	Infected Children	1512
INFY	Healthy Children	834
IgA	Infected Children	5.83
IgA	Healthy Children	3.68

Discussion:

The results of this study show a significant prevalence of the E. histolytica parasite among children aged 1–5 years, with clear differences related to age, sex, and geographic region. The higher infection rate among females may indicate differences in exposure or sex-related behaviors that influence susceptibility to the parasite. The increased infection rate in older age groups (5–6 years) also reflects the likelihood of increased exposure to environmental pollutants with age.

The geographic differences in infection rates, particularly the higher rates in the Baiji area compared to Tikrit, may be related to environmental factors such as water pollution or differences in hygiene and sanitation practices between regions. On the other hand, the variation in infection rates over the months, with a peak in July and a sharp decline in September, may reflect the influence of seasonal factors such as temperature, rainfall, or water usage patterns.

The elevated levels of INFY and IgA in infected children reflect a strong immune response to the infection. This indicates the body's attempt to fight the parasite, and these indicators may play a role in assessing the severity of the infection and monitoring the effectiveness of treatment. It should be noted that these findings necessitate a health intervention focused on improving public hygiene, providing safe drinking water, and promoting awareness to prevent the spread of this parasite, especially in high-risk areas.

Conclusion

This study highlights the significant prevalence of E. histolytica infection among children in Tikrit, emphasizing the need for enhanced preventive measures. The observed variations in infection rates based on age, gender, and geographic location suggest targeted intervention strategies. Future research should focus on evaluating the effectiveness of sanitation and health education programs to reduce the infection burden in these vulnerable populations.

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