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Isolation and Identification of Parasite Entamobea Histolytica and Giardia Lamblia from Euphrates River in AL- Nasiriyah City - Southern Iraq

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Abstract :The current study was conducted in four stations on the banks of the Euphrates River for a full year from September (2020) to August (2021) for the purpose of diagnosing parasites Entamobea histolytica and Giardia lamblia .Water samples were taken in clean and sterile plastic bottles and transported to the laboratory. The sedimentation method for parasites and the loglual ioden method were used to diagnose the parasite. Entamobea histolytica parasite was isolated from station (st1) with a percentage of 18%, and (st2) it accounted for 39%,in while station (st3)the precentage reached to 43%, Howeber station (st4) respictibely to 29%. The Giardia lamblia parasite was the least prevalent in the Euphrates River in station (st1), with apercent of 11.1%, in station (st2) reachd to 19%, but in station (st3) reached to25.6%, but in station (st4) was not found ,The results of the study also showed a concordance between air and water temperature in all study stations, the air temperature ranged between (37-16)°C and the water temperature ranged between (32-17)°C ,The statistical ftanalysis showed that there were significant differences ((P<0.05) between water and air temperatures in the four stations according to the months of the year.

Keywords: parasites Entamobea histolytica, Giardia lamblia, Temperature air and water

1. INTRODUCTION

Water plays a role in the transmission and spread of many different pathogens, as water contains microorganisms such as bacteria, viruses and parasites. Pathogens reach the water through human and animal waste Scientists believe that 80% of diseases in developed and non developing countries are caused by polluted water and the lack of procedures that contribute to water sterilization, and the Health Organization estimates that water pollutants cause approximately 250 million injuries annually and 10 million deaths (WHO,2006).
Reports in India, Australia, Bangladesh, Italy, Turkey, Iran, North America and South Africa have proven that humans are infected with this type of Entamoeba histolytica (Fotedar *et al*., 20078) and the rate of infection of these parasites, which increases (by an increase in many factors). Health awareness, hygiene, water pollution with human and animal waste, the spread of vector insects such as flies and the presence of domestic animals that are in contact with humans. For the growth and spread of parasites in their various stages (Al-Sumaidai, 2012), while the World Health Organization WHO(1997) indicated The parasite Entamoeba

histolytica is responsible for the death of more than 100,000 people annually, and the infection occurs as a result of ingestion of food and water contaminated with the cyst(Fletcher *et al*., 2012).
Giardia and *Cryptosporidium* are also parasites that are transmitted through contaminated water and are responsible for diarrhea and have direct life cycles consisting of an infective stage (sacs or eggs) that are resistant to environmental conditions and infection occurs as a result of ingestion of the infectious stages (Fletcher *et al*., 2012)The Giardiasis is one of the water borne parasitic diseases spread all over the world (Zakai & Barnawi, 2014). It was found that the spread of Giardiasis disease in population centers where it is transmitted through parasite cysts in contaminated water. These bags have the ability to survive for several weeks in cold water. As well as its ability to resist sterilization with chlorine (Sakhel, 2016)

2. MARERAL AND METHODS

1-2 The study Area

The Euphrates River is one of the most prominent rivers in Southwest Asia, its length is about 2,800 km, and what extends inside Iraq represents about 35% of the total river length (UNESCO, 2002) and it is classified among the longest rivers in the world. The South (Al-Saadi, 2006),the(station1) It is the area where the river enters the city of AL- Nasiriyah, where this station is located in the northwest of the city of AL-Nasiriyah, which is called the Sharif area and its coordinates(N 604806.867372;E 604806.867372,(station 2) This station is located in the middle of the river, it is located near Al-Nasr Bridge, which is(1400) km away from the first station, as it contains the buildings on the opposite side, and is considered the most polluted, as quantities of untreated sewage are poured and contains different organic waste, coordinates(N 3435118.8333;E 618633.067106,(station3) This massacre station is located At a distance of 4 from the second station, where there is pollution in this station and is considered the most polluted with organic pollutants Where large quantities of untreated sewage are poured into it, which contains various organic waste, and a sewage disposal station is located on the river (N 343405.50199 ; E 622538.854084 ,(station 4) This station is located in the southeast of the city of AL- Nasiriyah, which is the beginning of the river leaving the (5.300)km city. It is far from the third station. It is characterized by the presence of agricultural lands where field crops are grown and contains many aquatic plants (reeds and papyrus) as it is found in these coordinates(N 3429438.61464 ; E 625310.10546). Figure (1)

2-2 environmental examination

Temperature

Both water and air temperatures were measured using a graduated mercury thermometer from (0-100) degrees Celsius, and the measurement results were expressed in degrees Celsius. water directly

3-2 Sample collection

Water samples were collected for laboratory tests in (1)liter plastic bottles with tight stoppers. They were transferred to the laboratory of the College of Education for Pure Sciences / Thi Qar ,and the Technical Institute / Thi Qar within 2-3 hours to perform the necessary tests (APHA, 2003), River samples were collected Euphrates twice a month by immersing the collection bottle in water to a depth of (20) cm so that the direction of the bottle mouth is against the direction of the current, then remove the bottle cap to fill with water with the need

to leave a vacuum in the bottle after that the is closed directly and samples are collected simultaneously to ensure stability Environmental factors affecting the sample.

4-2 Parasitological examination

1- Each sample was filtered using a Buchner funnel and using a filter paper with a diameter of (0.45) µm. The filter paper was removed ,and placed (10 ml of filtered water), and each sample was placed in a sterile plastic tube (Test tube) in a centrifuge for 10 minutes at a time. At 2500 rpm, after centrifugation, the filtrate was discarded and the precipitate was taken for microscopic examination and parasite viewing.

2- Using iodine dye (loglual ioden) examined with a light microscope using a magnification power (40X) (Obiajuru & Adogu, 2013).

3. RESULTS AND DISCUSSION

1-3 Temperature air and water

Temperature is one of the influencing physical factors that depend on seasonal and local changes. It plays an important role in its impact on water bodies. It also affects the pH, salts, electrical conductivity and dissolved oxygen in the water. It also affects the distribution of living organisms (Al-Musleh, 1988)

Table (1,2) shows the monthly changes in water and air temperature at the study site. The air temperature ranged between (37-16) °C in December and August, where the highest value of air temperature reached to (37) in August, and the lowest value was (16) °C, and the statistical results showed that there were significant differences ($P<0.05$) between the air temperatures in the four stations according to the months of the year.

These results agreed with reached by (Al-Abadi, 2015) that the monthly changes amount to (35) °C, in the month of June, and also they agreed with (Ibrahim, 2019), The highest temperature reached to(35) °C in the months of July and August, and the lowest value was (15) °C in January, as it agreed with (Al-Asadi, 2014), It recorded that the highest value reached to(38) °C, and the lowest value reached to(15) in December, and it did not agree with the results of (Al-Sahlani, 2019) where it was found that the highest value reached (45) °C, July ,and the lowest value was (17) °C n February, It also did not agree with the results of (Turkih, 2018) where the highest value reached to (52)°C n July ,and the lowest value was (15)°C in February.

As for the water temperature as between(32-16)°C n August ,and January, where the highest value reached to (32)°C in August, and the lowest value reached to (16)°C January in the station (st1), while at the station (st2) As for the station (st2) where the highest value reached to(32)°C in the month of July, and the lowest value was (17) °C in January,

While the station (st3) reached the highest value of (31)°C in the months of June and July, and the lowest value of (18) °C in the month of January,

While station (st4) where the highest value reached (31)°C in July, and August, and the lowest value was(17)°C in December and January.

The statistical analysis showed that there were significant differences ($P<0.05$) between the water temperatures in the four stations according to the months of the year.

The results agreed with what was reached by (Al-Qaruni, 2011) which recorded that the highest water temperature in summer which amounted to (32)°C, and the lowest value in winter was (12)°C,it also agreed with (Sharhan, 2018) where the highest value of water temperature was recorded in Summer (36)°C, and the minimum value in winter (10)°C also agreed with the results of (Al-Yacoub, 2012) which recorded the highest value of water temperature (32.5) C in July, and the lowest value reached to (11)°C January, It also agreed

with the results of (Khithi, 2008) which recorded the highest value of (34)°C July, and August, and the lowest value is (17) C in December.

These results did not agree with what was reached by (Al-Abadi, 2015) which recorded the highest water temperature of (45)°C July ,and the lowest value (12.5)°C January, and it did not agree with the results of (Turkish, 2018 record) The lowest value was (12.5))°C in January, and the highest value was (46)°C July.

It was noted that the temperature has a wide range of monthly and local changes, and these differences are due to the differences in the prevailing climate in the region in summer and winter, and through the results of the study it was found that the water temperature follows the air temperature as the water, and air temperatures varied according climate during the season, It is a reflection of Iraq's climate, which is hot and dry in the summer and cold and rainy in the winter, and this difference is attributed to the time of sampling during the day, and the increase in the speed of water flow leads to a good mixing of water components, and then homogeneity of heat from the bottom to the surface (AI-Lami *et al*., 2001& Al-Budairi ,2012).

Table (1) Monthly changes of air temperature (°C)

station / month	Septmber2020	October 2020	November 2020	Desmber 2020	January 2021	February 2021	March 2021	April 2021	May 2021	June 2021	July 2021	August 2021	Site impact
St1	**30**	**29**	**19**	**16**	**20**	**26**	**29**	**32**	**36**	**36**	**36**	**37**	**29**
St2	**32**	**31**	**27**	**20**	**21**	**27**	**24**	**33**	**35**	**35**	**34**	**35**	**29**
St3	**30**	**26**	**24**	**20**	**24**	**28**	**21**	**30**	**33**	**34**	**33**	**33**	**28**
St4	**30**	**26**	**25**	**21**	**24**	**28**	**23**	**29**	**33**	**34**	**32**	**34**	**28**
Average monthly changes	**30**	**28**	**24**	**19**	**22**	**27**	**24**	**31**	**34**	**35**	**34**	**35**	**28**
LSD													
LSD=4.924 Effect (month and month					**Statiom LSD= 1.421**				**LSD= 2.462 month**				

station / month	Septmber2020	October 2020	November 2020	Desmber 2020	January 2021	February 2021	March 2021	April 2021	May 2021	June 2021	July 2021	August 2021	Site impact
St1	**27**	**27**	**19**	**18**	**16**	**21**	**25**	**28**	**32**	**30**	**30**	**32**	**25**
St2	**27**	**25**	**23**	**18**	**17**	**22**	**21**	**27**	**31**	**30**	**32**	**30**	**25**
St3	**25**	**23**	**23**	**20**	**18**	**23**	**22**	**24**	**28**	**31**	**31**	**30**	**25**
St4	**26**	**25**	**24**	**17**	**17**	**24**	**22**	**24**	**30**	**30**	**31**	**31**	**25**
Average monthly changes	**26**	**25**	**22**	**18**	**17**	**23**	**23**	**26**	**30**	**30**	**31**	**31**	**25**

LSD		
LSD=5.503 Effect (month and month	**Statiom LSD= 1.569**	**LSD= 2.752 month**

Table (2) Monthly changes of water temperature (°C)

2-3 diagnosis of parasites

In the present study, in which samples were collected from the Euphrates River for the period from September (2020) to the end of August (2021), when *Entamoeba histolytica, Giardia lambia, Entamoeba histolytica,* was found to reach the highest proportions of the parasite Entamoeba histolytica ,

Entamobea histolytica appeared in (st1), where 27 samples were collected, which diagnosed (5) samples with a percentage of 18.5%, while (3) samples appeared from *Giatia lamblia* with a percentage of 11.1% and in, (st2) collected 72 samples appeared samples (28) of *Entamobea histolytica* with a percentage of 38.9%, while the number of *Girdia lamblia* was(14) with a percentage of 19.4 %, while (82)samples were collected in (st3) 35 samples diagnosed from *Entamobea histolytica* with an appearance percentage of 42.7%, and *Giardia lamblia* (21) diagnosed with 25.7% appearance, and in station (st4) collected 7 sample from *Entamobea histolytica* appeared (2) with an appearance rate of 28.6% Giardia lamblia parasite was not found,There are many researches on evaluating the pollution of water sources. The results of the current study agreed with the results of (Entsar *et al*., 2016), It was noted that the infection rate in people who use river water as a source of drinking increased by 34.5%, while the percentage was lower in people who use well water as a source of drinking 0.9 %The results of the current study agreed with the study of Chazal & Adi, 2007) that the *Entamobea histolytica* was found with a percentage of 27.81%.

The reason for the high rate of infection in the river water with these parasites may be attributed, especially in the station site (st2) and (st3) is that the river water is subject to throwing human waste that is full of infectious stages of the parasite and animals, It may be used by humans without treating this properly, and untreated sewage water (Al-Kubaisi, 2000), It is noted through the results and similar studies that the parasites *Entamobea histolytica* and *Giardia lamblia* have the highest prevalence rate in the Euphrates River, Perhaps the reason for the spread of these two parasites is that they are parasites that have a simple life cycle, and their spread is through contaminated water and food or through oral-fecal transmission, and they have some distinctive characteristics that help their spread easily, and are not related to vector factors such as insects, as in Malaria and leishmaniasis (Esfandiari *et al*., 2002).

3-3 Parasites relationship with temperature

Figure (1) Temperature has an important role in chemical reactions and affects the dissolution of gases, and the relationship is direct with carbon dioxide CO2 and inversely with oxygen O2, There is a direct relationship between the increase in water temperature and parasites, and an inverse relationship between the temperature of the water and the diversity of parasites on the hostAlso, the drop in temperatures in winter has a direct effect on the growth of parasites Where the activity of parasites decreases as a result of a decrease in metabolic and enzymatic processes (Al-Sahlani, 2019), It was found that some parasites are resistant to high temperatures, which affect the parasites with free-living stages, Some parasites multiply at temperatures within the range (26-38) °C ,Whereas, a temperature of less than 26 °C leads to a slowdown in the growth and reproduction of parasites, while a temperature of more than 28 °C leads to death or hibernation of the parasitic stages (Al Emirate, 2019).

Where the most parasites appeared in the (st1) of the parasite *Entamoeba histolytica* in the month of April, which amounted to (2), and the water temperature ranged (38) ° C, while the most appearance of the parasite *Giardia lamblia* in the month of September was (2), as it reached Water temperature (27) °C, While the most parasites appeared in (st2), *Entamoeba histolytica* in a month In November, its number was (4) with water temperature (23) °C, As for the parasite *Giardia lamblia* it was found in the month (September, October, February, March, April, June) and its preparation was (2), as the water temperature values ranged between (21-30)°C, In (st3), the parasite *Entamoeba histolytica* appeared in the months of (October, April), where its number reached (5), an
d the water temperature values ranged between (25-31)°C, and the most frequent appearance of *Giardia lamblia* was in the month (November), Its number was (4), and the temperature of the water ranged from (23) °C, While parasites were found in station (st4), the parasite Entamoeba histolytica was found in the months of (March and May) and its number was (1), where the water temperature values ranged (21-31) °C , While parasites were found in station (st4), the parasite Entamoeba histolytica was found in the months of (March and May) and its number was (1), where the water temperature values ranged between (21-31) °C. As for Giardia lamblia parasite, it was not found As for Giardia lamblia parasite, it was not found.

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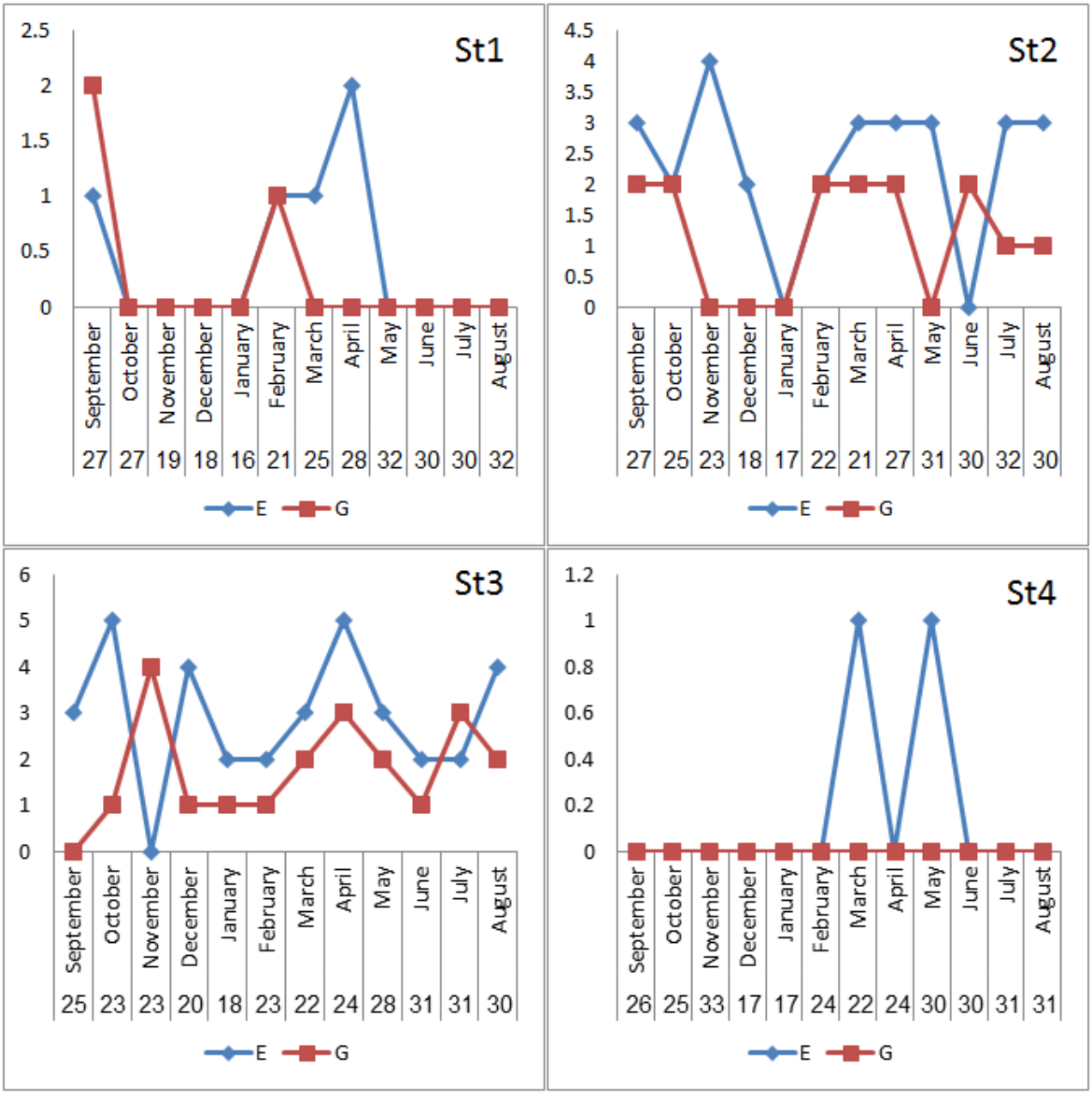

Figure (1) Parasite relationship with water temperature

The mature cyst of *Entamoeb histolytica* is surrounded by a thick wall composed of chitin, which gives resistance to unfavorable environmental conditions (Arredon *et al*., 2014), The accumulating phase is characterized by its ability to resist heat, drought, and chemicals such as fluorides, and is not affected by chlorination, which is used for the purpose of water sterilization (Schlegel & Jannasch, 2013), This phase is also characterized by survival and resistance to freezing and injury for a period of days, weeks or months when the appropriate humidity is available (Hamza, 2017),

It was also observed that the parasites of *Entamoeba* spp. It lives in water for two days at a temperature of(37)°C ,While it remains for 9 days at a temperature of (22) °C, At zero degrees Celsius, it lasts 60 days, While it is killed in dehydration, and on the other hand, it

was found that Giardia lamblia parasite can survive for a year at room temperature, while it kills the bagged phases at a temperature of(72) °C (Al-Hadithi, 2000)
The cystic phase of the parasite *Giardia lamblia* is characterized and the cyst phase, which is the phase that is resistant to environmental conditions and which is found in the environment (khalil *et al*., 2014), It is the carrier phase of the disease and it can remain in water and humid environments for several months because it has a strong cover that protects the cell from low pressure (Al-Sumaidi, 2012), and it can also live in water with low temperatures for several months (Tauma, 2018)

4. CONCLUSIONS

●The Euphrates River is more polluted than the Tigris River, and this is due to the discharge of sewage, animal excreta and human waste
●The possibility of using parasites as life evidence for the presence of fecal contamination in river waters
●Variation and spread of parasites in river water due to fecal contamination
●Significant differences were observed in the spread of parasites in the Euphrates River, where *Entamoeba histolytica* was the most prevalent during the study period

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