Prevalence Study of Major Protozoa Diarrheal Agents among Patients in Babylon Province Using Microscopically and Molecular Methods

Ahmed Khudhair Al-Hamairy [1*], Abid Ali Abeid Abiess [2] , Fadhil F.M.Aljoborae [3]

and Rana Salah Noori [4]

[1] Anesthesia Techniques Department, College of Health and Medical Techniques, Al-Mustaqbal University, 51001, Babylon, Iraq

[2] Medical Laboratory Techniques Department, College of Health and Medical Techniques, Al-Mustaqbal University, 51001, Babylon, Iraq

[3] Intelligent Medical System Department, Al-Mustaqbal University, 51001, Babylon, Iraq

[4] Chemistry Dept. College of Science for Women ,University of Babylon, Babylon, Iraq

Abstract:

From October 2019 to February 2020, 987 stool samples were examined using the direct smear method (Lugol's Iodine, Normal Saline [0.9%]) for the detection of the following parasites: *G. lamblia* and *E. histolytica*, while using floatation methods and the Ziehl–Neelsen method (Malachite green) for detecting *Cryptosporidium spp.* by light microscope. Ninety-six positive samples from these were examined by PCR for patients with diarrhea (children and adults, males and females) who visited the Babylon Maternity and Children's Hospital, the Babylon Province's Specialized Marjan Hospital for Internal and Cardiac Diseases, primary health care centers, and private clinics. The ages ranged from less than a year to 31 years and up.

Infection with parasites that cause diarrhea was found in 47.3% of the cases (*E. histolytica*, *G. lamblia*, and *Cryptosporidium spp.*), with rates of 26.4%, 17.9%, and 3.7%, respectively, in the current investigation. These were analyzed using a direct smear approach to detect the parasites' trophozoite, cyst, and oocyst phases.

By microscopic examination, the rural area had the highest rate of infection at 67.2 percent, compared to 32.9 percent in the metropolis. Males had the greatest infection rate of 51.5 percent, compared to 41.2 percent for females. The highest infection rate was observed at 76.10 percent in the age group (16-20), while the lowest infection rate was documented at 22.80 percent in the age group (26-30). Significant differences in infection rates have been observed at ($P \leq 0.05$).

In this study, 96 positive results in direct smear methods were employed to diagnose the major parasitic diarrhea agents using polymerase chain reaction (PCR). The total infection rate was 43.4 percent at the time (31.3 percent, 28.1 percent, and 2.1 percent, respectively). According to the PCR technique, males had the highest rates of infection (36.7 percent), while females had the lowest rates of infection (30.6 percent). In comparison to the rate of infection in urban regions, which was 25.9 percent, the highest rate of infection was found in rural areas (45.3 percent). In the current study, the highest rates of infection were found in the (16-20 years) age group (46.2 percent), while the lowest rates of infection were found in the (21-25 years) age group (16.7 percent).

Based on microscopic examinations and PCR methods, the new study indicated that parasites causing diarrhea are prevalent in Babylon province, and that rural areas had higher rates of infection compared to urban areas.

Key words : Microscopic inspection, PCR technique, *E.histolytica, G.lamblia*, and *Cryptosporidium* spp.

INTRODUCTON

One of the most common causes of "death" in developing countries and the second highest cause of death among newborns worldwide is diarrhea (World Health Organization). According to the World Health Organization, diarrhea can be caused by a range of pathogens, including bacteria, fungi, viruses, protozoa, or helminths, found in polluted food and drink (Vargas et al.). Entamoeba histolytica, Giardia lamblia, and Cryptosporidium sp. are three common protozoan infections that cause evolving diarrhea (Stark et al.). Epidemiologic risk factors include recent travel, crowded living conditions or attendance at a day-care center, consumption of raw or inadequately cooked foods, handling animals, contact with sick people, recent consumption of medications, underlying diseases such as HIV, and lack of vaccinations. Examination of stool specimens under the microscope for the presence of helminth eggs and protozoan trophozoites and cysts remains the gold standard for laboratory diagnosis of intestinal parasitic infections. Some parasite species (such as E. histolytica, G. lamblia, and Cryptosporidium sp.) cannot be detected by microscopy alone, while others may require the intervention of highly trained professionals (Fotedar et al.). PCR, also known as "polymerase chain reaction (PCR)" or "multiplex polymerase chain reaction (PCR)," is defined as "the amplification of multiple sections of DNA templates at the same time by adding more than one primer pair to the amplification reaction mixture immediately after its formation" (Verweij et al.; Haque). Using direct smear methods and polymerase chain reaction, the researchers hope to determine the relationship between these parasites and some factors (such as area of residence, gender, and age groups), as well as compare the prevalence of E. histolytica, Giardia lamblia, and Cryptosporidium sp. in fecal samples among patients who participated in the study.

MATERIALS AND METHODS

"Normal Saline Solution (Physiological)"

To make a 0.9% solution, dissolve 0.90 gm of NaCl in 100 mL of pure water, then autoclave at 121 degrees Fahrenheit and 15 pounds per square inch (PSI) for 15 minutes. Store at 4 degrees Fahrenheit until use (Garvery et al.).

Iodine by Lugol

prepared by dissolving five grams of KI in one liter of distilled water and slowly adding five grams of iodine crystals while swirling until they dissolve. Label the solution and store it in airtight containers (Zeibig, 1997).

"Carbol Fuchsin - Dimethyl Sulfoxide (CF-DMS) Stain"

- Dissolve fuchsin crystals in 25 mL of ethyl alcohol to make a 99% solution.
- After liquefaction in a water bath, add 2 g of phenol crystals and stir thoroughly with a glass rod.
- Add 25 mL of pure glycerol, 25 mL of "dimethyl sulfoxide (DMS)," and 75 mL of distilled water, and mix thoroughly.
- Dilute 220 mL of 2% malachite green in distilled water as a clearing solution.
- Filter the solution through Whatman filter paper No. 4 after letting it stand for up to 30 minutes.

- The stain can be used immediately or stored in a dark, room-temperature bottle for later use

Method of Direct Wet Mounting

A little drop of "normal saline" (0.9%) or "iodine stain" was added to the slide glass and thoroughly mixed with a small piece of stool, after which cover slides were applied, and the sample was studied under magnifications of 40X and 100X (Tanyuksel& Petri, 2003). A direct smear approach was used on clean glass slides to evaluate feces samples collected.

Floatation Techniques

The following procedures were used to isolate oocysts from feces: The feces were suspended in PBS (pH 7.2) in a 20:80 ratio and centrifuged at 500g for 10 minutes to obtain the final concentrations. In 15 mL centrifuge tubes filled with PBS (pH 7.2), 3-5 mL ether, and vigorous shaking, the sediment was suspended in the supernatant and discarded. From the bottom to the top, four layers were separated by centrifugation at 500g for one minute: ("1-Sediment, 2-PBS, 3-Debris, and 4-Solvent"). The sediment layer, which included 75% of the oocysts, was used to purify the oocysts after they had been harvested. They were produced in 2.5% K2Cr2O7 (Arrowood M J, and Sterling, 1987) until they were used in the experiment.

Staining Procedure

- A sterile wooden stick with a cotton head was used to make a rectal fecal smear on a clean glass slide that was then left to dry at room temperature.
- Methanol was used to fix the dry smear for 5-10 seconds (sec.).
- 5 minutes of staining with CF-DMS
- 10-30 seconds of washing with tap water
- The smears were rinsed for 1 minute in Malachite green (2%) to create a green background.
- The smears were washed with tap water for 10 seconds.
- The smears were left to dry for ten minutes.
- The smear was lubricated with oil immersion using a wooden stick.
- Oil objectives of 40X and 100X were used to examine the smears under a light microscope.

Ziehl–Neelsen method has been modified.

Following are the procedures that were followed for the examination of rectal smears using the modified Ziehl-Neelsen stain MZN-ST (acid fast). The excrement stain was made permanent for 5 minutes with methanol alcohol, and then allowed to dry at ambient temperature to complete the process. The dehydrated smears were then stained for an hour with Carbol Fuchsin, which was prepared by dissolving 15 percent Carbol Fuchsin in methanol and adding it to the dehydrated smears (stock solution). In 90 mL 5 percent phenol, dissolve 10 mL Ziehl Fuchsin. Following that, I rinsed with regular tap water. After that, agitate the slide for 20 seconds while diffusing a 2 percent H2SO4 solution onto the surface of the slide. Rinsing was accomplished using ordinary tap water. It was then stained for five minutes with a 5 percent malachite green solution, washed with tap water, and dehydrated to complete the procedure. The samples were then examined with oil immersion objectives at 40X and 100X magnifications (Jawtez *etal*., 2001).

Polymerase chain reaction multiplex (PCR)

Multiplex PCR was used to detect the presence of "*G. lamblia*, *E. histolytica*, and Cryptosporidium sp. based on subunit ribosomal rRNA genes" in human stool samples using the Multiplex PCR technique. For the purpose of carrying out this procedure, the following actions were taken:

Extraction of Genomic DNA

DNA from feces samples was extracted using the AccuPrep® Stool DNA Extraction Kit from Bioneer Korea, which was used in accordance with the manufacturer's instructions:

- 200 mg of single positive stool samples from "G. lamblia, E. histolytica, and Cryptosporidium sp." were moved to a sterile 1.5 ml microcentrifuge tube, and then 20 l proteinase K and 400 l Stool lysis buffer (SL) were added and mixed by vortexing together. Incubation at 60 degrees Celsius for ten minutes followed by centrifugation at 12,000 rpm for five minutes completed the procedure.
- The supernatant was transferred to a fresh tube, and 200 l of binding buffer was added to each tube individually.
- Incubate the tubes at 60°C for another 10 minutes.
- The samples were mixed by lightly vortexing for five seconds and then spinning down for ten seconds to remove any liquid that had adhered to the tube's sides and cover. A total of 100 l of isopropanol was added.
- When the DNA filter column was placed in a two-ml collection tube, it was possible to transport the entire mixture (including any precipitate) to the column at one time. Following that, the sample was centrifuged for five minutes at 8000 rpm. This was done by discarding the 2 ml flow-through collection tube and placing the column in another 2 ml flow-through collection tube.
- 500 ml W1 buffer was added to the DNA filter column, and it was centrifuged for 30 seconds at 10000 rpm. The flow-through was removed and a new one was installed in its place; return the column to the two-ml collecting tube.
- Each column received 500 liters of W2 Buffer (ethanol). Then centrifuged for 30 seconds at 8000 rpm. The flow-through was discarded, and the column was reinserted into the two-milliliter collection tube.
- To dry the column matrix, all of the tubes were centrifuged for one minute at 12000 rpm.
- It was transferred to 1.5 ml microcentrifuge tubes, and 50 l of pre-heated extraction buffer was added to the center of the DNA filter column matrix.
- The elution buffer was allowed to stand in the tubes for at least five minutes to ensure that it was absorbed by the matrix. Centrifugation at 10000 rpm for 30 seconds eluted the pure DNA.

DNA Genomic Profile

The purity of extracted genomic DNA from feces samples (96 samples) for all parasites was examined using a nanodrop spectrophotometer (THERMO, USA), which checks and measures DNA purity by reading the absorbance at (260/280 nm) as follows:

- After launching the nanodrop software, select the appropriate application "(Nucleic acid, DNA)".
- Dry wipes were used numerous times to clean the measurement pedestals. Then carefully pipette one microliter of water onto the bottom surface of the measurement pedestal.
- The sample arm was lowered, the Nanodrop was initialized by clicking OK, and the pedestals and one l of the suitable blank were cleaned.
- In addition to the DNA samples, the same elution buffer (blanking solution) was used.

- One microliter of DNA is pipetted onto the pedestals, and the results are analyzed. Pure DNA can be extracted when the absorbance ratio is high enough (1.8).

Primers

The current study produced three PCR primers based on the subunit ribosomal rRNA gene to detect "G. lamblia, E. histolytica, and Cryptosporidium sp." using "NCBI-GenBank (M54878.1, X64142.1, and AF112573.1, respectively)" and three primers plus online design. As may be seen in the table below, Bioneer, a Korean company, provided these primers:

PCR Size	Sequence	Primer	
574 bp	**GTTGAAACGCCCGTAGTTGG**	**F**	***G.lamblia***
CTCGCTCGTTGTCGCAATG		**R**	
204 bp	**ACGAGGAATTGGGGTTCGAC**	**F**	***E.histolytica***
CACCAGACTTGCCCTCCAAT		**R**	
351 bp	**AACCTGGTTGATCCTGCCAG**	**F**	***Cryptosporidium. sp***
TTCCCCGTTACCCGTCATTG		**R**	

Preparation of a multiplex PCR master mix

The AccuPower® Gold Multiplex PCR PreMix Kit was used to make the PCR master mix, which was done according to the company's instructions as shown in the table below:

Volume		Multiplex PCR Master mix
5µL		**DNA template**
1µ L	**G.L- F**	**Forward primer (10pmol)**
1µ L	**E.H- F**	
1µ L	**C.sp.- F**	
1µ L	**G.L- R**	**Reverse primer (10pmol)**

1µ L	E.H- R	
1µ L	C.sp.- R	
39 µ L		PCR water
50 µ L		Total volume

PCR thermocycler conditions by using convential PCR thermocycler system as following table

repeat	Time	Temp.	PCR step
1	5min	95C°	Initial Denaturation
30 cycle	30sec.	95C°	Denaturation
	30sec	58C°	Annealing
	1min	72C°	Extension
1	5min	72C°	Final extension

Analyze the PCR product

The following steps were used to examine the PCR results using agarose gel electrophoresis:

- A 1.5 percent Agarose gel was made by dissolving 1X TBE in a water bath at 100 °C for 15 minutes, then cooling to 50 °C.
- The ethidium bromide stain was then applied to the agarose gel solution three times.
- The comb was carefully removed from the tray and 10 l of PCR product and 5 ul of (100bp Ladder) were added to each comb well after the agarose gel solution was placed in the tray and allowed to harden for 15 minutes at room temperature.
- The electrophoresis chamber was filled with 1X TBE buffer before the gel tray was placed inside. After that, for an hour, a 100 volt, 80 degree Fahrenheit electric current was used to illuminate five PCR products with a UV transilluminator, followed by digital photography of the bands revealed (Samsung, China).

Results and Discussion

987 stool samples from patients with diarrhea were analyzed in the new study. DNA was isolated from the positive samples and used in multiplex polymerase chain reaction (PCR) for "G. lamblia, E. histolytica, and Cryptosporidium sp.", and then the samples were examined under a light microscope.

Table 1: Percentage of infection for parasites that cause diarrhea according to residence area by using direct smear method

Residence area	Examined No.	Infected No.	Parasites that cause diarrhea						Total percentage of infection (%)
			E.histolytica		*G.lamblia*		*Cryptosporidium* spp.		
			Infec. No	(%)	Infec. No	(%)	Infec. No	(%)	
Urban areas	572	188	97	17	75	13.1	16	2.8	32.9
Rural areas	415	279	164	39.5*	102	24.6*	21	5.1*	67.2*
Total	987	467	261	26.4	177	17.9	37	3.7	47.3
Statistical analysis (Z-test)									

*Significant differences ($P \leq 0.05$)

Table 2: Percentage of infection for parasites that cause diarrhea in according to the sex by using direct smear method.

Gender	Examined No.	Infected No.	Parasites that cause diarrhea						Total percentages of infection (%)
			E.histolytica		*G.lamblia*		*Cryptosporidium* spp.		
			Infec. No.	(%)	Infec. No.	(%)	Infec. No.	(%)	
Males	584	301	178	30.5*	103	17.6	26	4.5*	51.5*
Females	403	166	83	20.6	74	18.4*	11	2.7	41.2
Total	987	467	261	26.4	177	17.9	37	3.7	47.3
Statistical analysis (Z-test)									

*Significant differences($P \leq 0.05$).

Table 3:Percentage of infection for parasites that cause diarrhea in according to the age groups by using direct smear method.

Age groups	Examined No.	Infected No.	Parasites that cause diarrhea						Total Percentages of infection (%)
			E.histolytica		*G.lamblia*		*Cryptosporidium* spp.		
			Infec. No.	(%)	Infec. No.	(%)	Infec. No.	(%)	
Less than one year	219	95	43	19.6	22	10	31	14.2*	43.4
1-5	126	63	36	28.6	26	20.6	6	4.7	50
6-10	191	76	44	22	37	19.4	0	0	39.8
11-15	168	104	65	38.7	40	23.8	0	0	61.9
16-20	113	86	48	42.5*	37	32.7*	0	0	76.1*
21-25	78	20	11	14.1	7	9	0	0	25.6
26-30	57	13	7	12.3	5	8.5	0	0	22.8
31and more	35	10	7	20	2	5.7	0	0	28.6
Total	987	467	261	26.4	177	17.9	37	3.7	47.3
Statistical analysis (LSD-test)									

*Significant differences ($P \leq 0.05$).

Table 4 : Incidence of infection according to the collecting sample monthly.

Months	Examined No.	Infected No.	Parasites that cause diarrhea						Total percentages of infection (%)
			E.histolytica		*G.lamblia*		*Cryptosporidium* spp.		
			Infec. No	(%)	Infec. No	(%)	Infec. No	(%)	
October 2019	116	74	52	44.8*	18	15.5	8	6.9*	63.8*
November 2019	178	93	49	27.5	37	20.8	9	5.1	52.2
December 2019	260	112	58	22.3	44	16.9	10	3.8	43.1
January 2020	243	135	72	29.6	57	23.5 *	6	2.5	55.6*
February 2020	192	53	30	15.6	21	10.9	4	2	27.6
Total	987	467	261	26.4	177	17.9	37	3.7	47.3
Statistical analysis (LSD-test)									

*Significant differences ($P \leq 0.05$)

Table 5 : Type of parasite infection quantity and Its rates in the Babylon province.

Type of infection	Infected No.	%	Type of parasites		
			The parasite	Infected No.	%
Single	459	98.3	*E.histolytica* *G.lamblia* *Cryptosporidium* spp.	253 169 37	54.2 36.2 7.9
Double	8	1.7	*E.histolytica* +*G.lamblia*	8	1.7
Triple	0	0	-	0	0
Total	467	47.3	-	467	47.3

Table 6 : Percentage of infection for parasites that cause diarrhea in according to residence area by using multiplex PCR technique.

Residence area	Examind No.	Infected No.	Parasites that cause diarrhea						Total percentages of infection (%)
			E.histolytica		*G.lamblia*		*Cryptosporidium* spp.		
			Infec. No	(%)	Infec. No	(%)	Infec. No	(%)	
Urban area	54	14	13	24.1	11	20.4	1	1.9	25.9
Rural area	42	19	17	40.5*	16	38.1*	1	2.4*	45.3*
Total	96	33	30	31.3	27	28.1	2	2.1	43.4
Statistical analysis (Z-test)									

*Significant differences ($P \leq 0.05$).

Table 7 : Multiplex PCR incidence of studied parasites infection according to sex of the patient.

Gender	Examind No.	Infected No.	Parasites that cause diarrhea						Total percentages of infection (%)
			E.histolytica		*G.lamblia*		*Cryptosporidium* spp.		
			Infec. No	(%)	Infec. No	(%)	Infec. No	(%)	
Males	60	22	21	35*	16	26.7	2	3.3*	36.7*
females	36	11	9	25	11	30.6*	0	0	30.6
Total	96	33	30	31.3	27	28.1	2	2.1	43.4
Statistical analysis (Z-test)									

*Significant differences ($P \leq 0.05$).

Table 8 : Multiplex PCR incidence of studied parasites infection according to age groups of patients .

Age group	Examind No.	Infected No.	Parasites that cause diarrhea						Total percentages of infection (%)
			E.histolytica		*G.lamblia*		*Cryptosporidium* spp.		
			Infec. No	(%)	Infec. No	(%)	Infec. No	(%)	

Less than one year	16	4	3	18.8	2	12.5	1	6.3	25
1-5	14	6	6	42.9	4	28.6	1	7.1*	42.9
6-10	16	6	2	12.5	5	31.3	0	0	37.5
11-15	17	7	9	52.9*	7	41.2*	0	0	41.2
16-20	13	6	6	46.2	5	38.5	0	0	46.2*
21-25	6	1	1	16.7	1	16.7	0	0	16.7
26-30	9	2	2	22.2	3	33.3	0	0	22.2
31and more	5	1	1	20	0	0	0	0	20
Total	96	33	30	31.3	27	28.1	2	2.1	43.4
Statistical analysis (LSD-test)									

*Significant differences ($P \leq 0.05$).

Table 9 : Multiplex PCR incidence of studied parasites infection according to the collecting sample monthly.

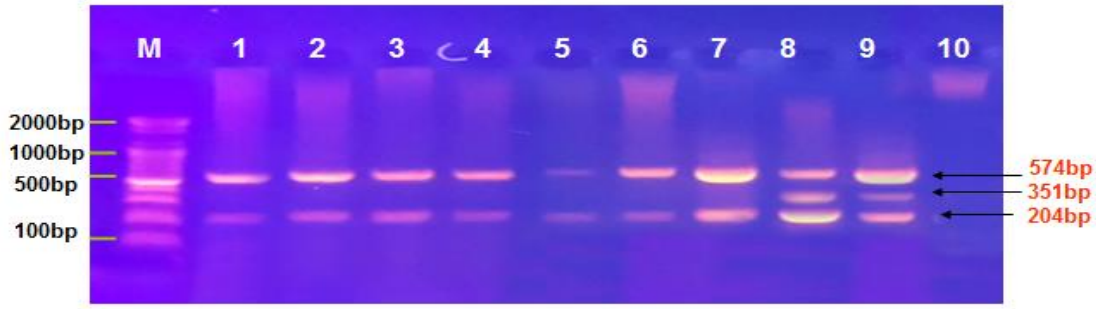
Months	Examind No.	Infected No.	Parasites that cause diarrhea						Total percentages of infection (%)
			E.histolytica		*G.lamblia*		*Cryptosporidium* spp.		
			Infec. No	(%)	Inecf. No	(%)	Infec. No	(%)	
October 2019	28	11	10	35.7	9	32.1	1	3.6	39.3

November 2019	**19**	**8**	**7**	**36.8***	**6**	**31.6**	**0**	**0**	**42.1***
December 2019	**13**	**3**	**3**	**23.1**	**3**	**23.1**	**0**	**0**	**23.1**
January 2020	**16**	**4**	**4**	**25**	**3**	**18.8**	**1**	**6.3***	**25**
February 2020	**20**	**7**	**6**	**30**	**7**	**35***	**0**	**0**	**35**
Total	**96**	**33**	**30**	**31.3**	**27**	**28.1**	**2**	**2.1**	**43.4**
Statistical analysis (LSD-test)									

***Significant differences ($P \leq 0.05$).**

Table 10 : type of parasite infection quantity and rates in the Babylon province.

Type of infection	Infected No.	%	Type of parasites		
			The parasite	Infected No.	%
Single	9	27.3	*E.histolytica* *G.lamblia* *Cryptosporidium* spp.	6 3 0	18.2 9.1 0
Double	22	66.7	*E.histolytica +G.lamblia*	22	66.7
Triple	2	6.1	*E.histolytica +G.lamblia +* *Cryptosporidium spp.*	2	6.1
Total	33	43.4	-	33	43.4



Figure(1): Agarose gel electrophoresis image that show the Multiplex PCR product analysis of ss-rRNA gene from genomic DNA of human stool samples. Where M: Marker (2000-100bp), lane(1-10) positive samples for *G.lamblia* at 574bp and *E.histolytica* at 204bp. Lane (8 -9) positive samples for *Cryptosporidium* spp. at 351bp product size. Lane (10) negative sample.

Using the direct smear method, the percentage of infection for parasites that cause diarrhea was analyzed according to residential area. According to the findings of this study, parasite infection rates differed significantly depending on where people lived in Babylon Province, with the rural area having the highest prevalence (67.2 percent) and the city center having the lowest frequency (2.4 percent) (32.9 percent). Baghdad had the greatest rate of infection, with 50.9 percent, according to this study, which verifies the findings of Al-Kubaisy et al. It's also in agreement with Al-Taie and Al-Mosa in Babylon Province, where the rate of infection in rural areas increased by 64.7 percent while the rate in urban areas declined by 35.3 percent (Al-Mussawi). More than seventy-nine percent more people become infected in rural areas and 15 percent in urban areas than in the city areas, according to Al-Mussawi (12.36 percent). According to the results of this study, which employed the PCR method to determine parasite infection rates by residential region, the rural area had the greatest infection rates (45.3 percent) and the city had the lowest infection rates (25.9 percent). Those findings are supported by Al-Khufaji, which indicated that the rate of infection was higher in rural areas (62.9 percent) than in metropolitan areas (37.9 percent). According to Al-Muhana, in Al-Najaf, the rural area had a greater infection rate than the metropolitan area (19.6 percent) (9.1 percent). Lack of clean water, reliance on river water directly as a source of water, lack of guidance and counseling by the authorities, as well as the rural population's lower health and cultural level, the lack of hospitals and health centers in those areas, and the use of animal waste and human feces as fertilizers are all factors that contribute to the high incidence of infection in rural areas. When the direct smear method is used, a patient's gender influences the percentage of infection for parasites that cause diarrhea. Men (51.5 percent) and women (47.3 percent) both had parasitic illnesses, with men having the highest percentage (51.5 percent) and women having the lowest number (41.2 percent). According to a study by Al-Kubaisy in Baghdad, males have the highest rate of infection (58.5 percent) and females the lowest rate of infection (48.5 percent) (41.5 percent). This agrees with Al-Murshdy's findings as well. According to his findings, men (53.9 percent) were more likely than women to become infected (47.8 percent). However, he has observed no significant differences between males and females in the studies by Jaeffer and Mahmud in Baghdad, and in Al-Sowera. Study areas (rural region, town) saw male infection rates of 18.14 percent and females had infection rates of 14.42 percent and 8.31 percent, respectively, according to Al-Mussawi. Also, in Babylon Province, there was a disagreement with Al-Mamouri. Compared to females, males had the highest risk of infection (80.6 percent) (81 percent). Men were the most likely to be infected (6.12 percent), while women were the least likely to be infected (5.11 percent), according to Al-Ibrahimi. Using the PCR technique, this study found that 43.4 percent of both sexes were infected with parasites, with males having the highest rate of infection (36.7 percent) and females having the lowest percentage (30.6 percent). There were no significant differences between men and women in the percentage ratios of males to females (14.98 percent and 14.58 percent, respectively) in the current study,

which concurs with Al-Warid in Baghdad but contradicts Koffi et al., which found significant differences between men and women despite the highest infection rate among women (65.36 percent) and the lowest infection rate among men (14.58 percent) (60.13 percent). As a result of their increased activity and exposure to external environmental factors and their role as a working group in their community, men may be more susceptible to the pathogens that sicken women because of their propensity to consume food and drink from street vendors, as well as their disregard for personal hygiene and hand washing. By employing the direct smear method, the percentage of infection for parasites that cause diarrhea was calculated for different age groups of patients. Different age groups, from newborns to adults, were studied to determine the prevalence of parasitic illnesses. At 76.1 percent, the group (16-20) was the most infected, followed by the age group (26-30)

CONCLUSION

There are several conclusions to be drawn from this study:

- The prevalence of parasites that cause diarrhea in Babylon province is very high when detected using a microscopic examination method and a polymerase chain reaction technique (PCR), and rural areas have higher rates of infection than cities.
- There was a close relationship between infection and sex, age group, and residence area.

Cryptosporidium sp. parasites had the lowest prevalence of all parasites, with illness primarily affecting children under the age of five.

ACKNOWLEDGMENTS

Many thanks and gratitude to the Department of Biology, College of Science for Women, University of Babylon and its laboratories, as well as the Medical Laboratory Techniques Department, College of Health and Medical Techniques, Al-Mustaqbal University, for conducting research and providing the required facilities.

- **REFERENCES**

Abdul-Hadi C, Al-Saadi H, Al-Hamairy A. 2016. Effect of the Experimental Infection with *Toxoplasma gondii* on some Biochemical aspects and Histological Changes for the Liver and Spleen in Female Rats. *International Journal of PharmTech Research*. 9(11): 142-150.

Al-Ameer Z, Al-Hamairy A, Omran D. 2020. Prevalence of Toxoplasmosis and Relationships With Estradiol Hormone and Vascular Cells Adhesion Molecular-1(VCAM-1). *Indian Journal of Forensic Medicine & Toxicology*. 14 (3): 1434-1439.

Al-Hamairy A. 2016. Effect of Lipopolysaccharide extracted from *Escherichia coli* on the growth and development of promastigote of *Leishmania tropica* (In vitro study). *International Journal of PharmTech Research*. 9(4): 377-386.

Al-Hasnawi, N.Z.S.S., Al-Hamairy, A.K., Altameme, H.J.M. 2020. Haplotyping of *Echinococcus granulosus* Isolates From Human, Sheep and Cattles Hydatid Cysts in Some Central Euphrates Provinces, Iraq. *Indian Journal of Forensic Medicine and Toxicology*. 15(3): 1418–1424.

Al-Hasnawi, N.Z.S.S., Al-Hamairy, A.K., Altameme, H.J.M. 2020. Evaluation activity of boiled aqueous extract of broccoli flower plant (*Brassica oleraceae* L. var. *italic*) and albendazole drug against the protoscolices In vitro. *Indian Journal of Forensic Medicine and Toxicology*. 14(4): 2460–2464.

Al-Ibrahimi LA. 2013. Molecular Detection of *Giardia intestinalis* In Stool of Children Infected With Diarrhea By Polymerase Chain Reaction. Some Hospitals in Al-Diwaniya City, M.Sc. Thesis, Education College, University of Al-Qadisiya.

Al-Khufaji AS. 2013. Molecular Characterization Of *Entamoeba moshkoviskii* As The First Recording At Al-Qadisyia Province Iraq By Using Single Round Polymerase Chain Reaction. MSc. Thesis College of Medicine, University of Al-Qadisyia.

Al-Kubaisy W, Al-Talib H, Al-Khateeb A, Shanshal M. 2014. *J Trop. Biomedicine*. 31: 499–506.

Al-Mamouri AK. 2000. Epidemiology of Intestinal Parasites and Head Lice Among Some Primary Schools Pupils In The Al-Mahaweel District, Babylon Province. M.Sc. Thesis, Science College, Babylon University.

Al-Mamouri SA. 2014. Evaluation Of Some Plant Extracts and Metronidazole Drug In vitro of *Entamoeba histolytica* Viability and Molecular Study For Some Species Determination In, Babylon Province. M.Sc. Thesis, College of Science for Women, University of Babylon.

Al-Muhana WH. 2013. Epidemiological And Diagnosis Study Of The *Giardia lamblia* Parasite Genotypes, Which Causes Diarrhea Among The Patients In Al-Najaf Al-Asharf, Province By Using the PCR Techniques. M.Sc. Thesis, Education College for Girls, University of Kufa.

Almuhana, W.H. Y. and Al-Hamairy, A. K. 2021. Incidence of *Toxoplasma gondii* and Relationship with Some Inflammatory Factors in Babylon Province. *Indian Journal of Forensic Medicine & Toxicology*. 15 (1): 1660-1671.

Al-Murshdy KA. 2007. *J Iraq Babylon University*. 1: 179-185.

Al-Mussawi HS. 2012. Epidemiological Study For *Giardia lamblia* Parasite In Babylon Province and Test Activity Of Cold Aqueous Extract And Crude Powder Of Pomegranate Peals In Experimentally Infected Cats, M.Sc. Thesis, College of Science for Women, University of Babylon.

Al-Taie HH, Al-Mosa AH. 2006. *J Alotaqani*. 5: 108-115.

Al-Warid HS, Mahmood SH, Al-Saqur IM. 2010. Study in Epidemiology and PCR Detection of Cryptosporidiosis in North of Baghdad. (Ph.D.) Thesis, Baghdad University, Science College.

Arrowood M J, and Sterling C R. 1987. *J Parasitology*. 73: 314-319.

Fotedar R, Stark D, Harkness J. 2007. *J Clin Microbiology*. 20: 511-532

Garvery J S, Cremer NE, Sussdorf DH. 1977. Method In Immunology, A Laboratory Text For Instruction and Research, 3rd edn., W.A. Banjamin, Inc. Canada, pp. 1-518.

Haque R. 2007. *J Health Popul Nutrition*. 25(4): 387–391.

Jaeffer HS. 2011. *J University of Anbar For Pure Science*. 5: 1-5.

Jawtez-Melnick S, Adeberg S. 2001. Medical Microbiology, 22nd edn., Large Medical Books, McGraw-Hill, pp. 1-582.

Koffi M, N'Djeti M, Thomas Konan T, Yao Djè Y. 2014. *J African Environ Sci Technol*. 8: 178-184.

Mahdi Z, Al-Hamairy A, Al-Rubaiey, H. 2020. Genotyping of *Echinococcus granulosus* Isolates from Human, Sheep and Cattles Hydatid Cysts in Some Central Euphrates Provinces, Iraq. *Medico-legal Update*. 20(2): 570-575.

Mahmud SA. 2006. *J Alotaqani*. 3: 129-138.

Manji ZF, Al-Hamairy AK. 2015. Seroepidemiological and Molecular Study of Toxoplasmosis in the Blood Donors and Applicants for Marriage peoples in the Babylon Province, Iraq. *Res. J. Pharm. Bio. Chem. Sci.* 6(6): 1106-1114.

Oliewi M, Al-Hamairy, A. 2016. Epidemiological and Diagnostic Study for Diarrheic Parasites (*Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium sp.*) Among Diarrheic Infected Patients By Using Multiplex Polymerase Chain Reaction in the Babylon province, Iraq. *Res. J. Pharm. Bio. Chem. Sci.* 7(1): 438-447.

Pour T R, Koyfman A, Runyon MS. 2013. *African J Emerg Medicine*. 3: 75–82.

Stark D, Al-Qassab SE, Barratt JLN, Stanley K, Roberts T, Marriott D, Harkness J, Ellis JT. 2011. *J Clin Microbiology*. 9: 257–262.

Tanyuksel M, and Petri W A. 2003. *Clin Microbiol Reviews*. 16: 713-29.

Vargas M, Gascon J, Casals C, Schellenberg D, Urassa H, Kahigwa E, Ruiz J, Vila J. 2004. *American J Trop Medicine*. 70: 536-539.

Verweij J J, Roy A, Templeton K, et al. 2004. *J Clin Microbiology*. 3: 1220–1223.

Victora CG, Bryce J, Fontaine O, Monasch R. 2008. *Bull WHO*. 73: 1246 – 1255.

World Health Organization. 2009. World health Report, Geneva, Switzerland, pp. 1-300.

Zeibig, E. A. 1997. Clinical Parasitology: A practical Approach. W. B. Saunders Co., Philadelphia, pp. 1-320.