Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 3 Vol 8 No 1 2023

Article

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

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Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.03.43

Abstract
Direct Smear Method (Lugol's Iodine, Normal Saline (0.9 percent) for detection of G.lamblia and E.histolytica while using floatation methods; Ziehn–Neelsen method (Malachite green) for detection of Cryptosporidium spp. by using a light microscope; 96positive sample from that sample were examined by "polymerase chain reaction (PCR)". Diarrhea patients at the Babylon maternity and children's hospital, the Babylon province's "specialized Marjan Hospital for Internal and Cardiac Diseases," primary health care facilities, and private clinics are all included in this category of patients. The children's ages range from (31 and up). Infection with parasites that cause diarrhea47.3 percent (E.histolytica, G.lamblia, and Cryptosporidium spp.) was found to be 26.4percent, 17.9percent, and 3.7percent, respectively, in the current investigation. They were analyzed using a direct smear approach to detect the parasites' trophozoites, cysts, and oocyst phases. By microscopic examination, the rural area had the highest infection rate at 67.2 percent, compared to 32.9 percent in the metropolis. Males had the most significant infection rate of 51.5 percent, compared to 41.2 percent for females. The higher infection rate was observed at 76.1% in the "age group"(16-20), while the lower infection rate was documented at 22.8 percent in the "age group" (26-30). Significant differences in infection rates have been seen at the ($P \leq 0.05$). This study employed 96 positive results in direct smear methods to diagnose the significant parasite diarrhea agent using polymerase chain reaction (PCR). It had a "total infection rate" of 43.4 percent (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%), while females had the lowest infection rates (30.6%). Compared to the infection rate in urban regions, which was 25.9%, it had the maximum infection rate in rural areas (45.3%). In the current study, the maximum infection rates were found in (the 16-20years) age group (46.2%), while the lowest rates of infection were found in the (21-25-year-old age group (16.7%). In contrast to previous studies, the present research has shown that the prevalence of diarrhea-causing parasites in the province of Babylon is higher when detected by microscopic examination and PCR, and the infection rate from urban to rural areas is high.

Keywords: Microscopic inspection, PCR technique, E.histolytica, G.lamblia, and Cryptosporidium spp.

Introduction

A leading cause of death in impoverished republics and the additional most significant cause of neonatal death in the globe is diarrhea[1]. The World Health Organization defines dairy as having more feces than usual or passing watery stools more than three times in twenty-four hours due to a range of microorganisms in contaminated food or beverages [2] Cryptosporidium sp. is one of the most common parasitic pathogens associated with diarrhea, although there are several others to consider [3,4]

These epidemiological risk factors [5] include recent portable, crowded living or daycare, raw or undercooked food consumption, animal encounters, ill contacts, and recent drug usage. Detecting helminth eggs and protozoan trophozoites and cysts in stool samples is still the primary method for determining the presence of intestinal parasites. Even with the most advanced microscopy, parasites including E.histolytica, G. lambla, and Cryptosporidium ps. can be challenging to detect [6] Multiple primer pairs are added to the amplification reaction mixture so that several different parts of the DNA can be multiplied simultaneously. And [7,8]. In this study, we tried to determine the relationship between these parasites and different aspects (residential area, sex, and age group) and compared "E.histolytica, G. lamblia, and Cryptosporidium sp." Using "direct smear and polymerase chain reaction methods" in stool samples of patients enrolled in this study.

Materials and Methods

Normal Saline Solution (Physiological)

To make the 0.9 percent solution, melt 0.90 gm sodium chloride in 100 ml pure water, then autoclave at 121 degrees Fahrenheit and 15 pounds / Lange for 15 minutes and store at 4 degrees Fahrenheit until use [9]

Iodine by Lugol

These iodine crystals were slowly swirled into five grams of a solution created by dissolving iodide crystals in water and then labeling and storing the solution in airtight containers. [10]

• Carbol Fuchsin - Dimethyl Sulfoxide (CF-DMS) stain

Fucshin crystals were dissolved in 25 mL ethyl alcohol to make the (99 percent).

• After liquefaction in a water bath, 2 gm. phenol crystals were added and stirred thoroughly with a glass rod.

• 25 mL pure glycerol, 25 mL "dimethyl sulfoxide (DMS)," and 75 mL (DW) were added and thoroughly mixed.

• 220 mL 2% malachite green diluted in distilled water as a clearing solution

• The solution was filtered on what man "filter paper" No. 4 after being left for up to 30 minutes.

• The stain could be used immediately or kept in a dark, room-temperature bottle for later use.

Method of direct wet mounting

Add a little salt (0.9percent) or "iodine stain" to the slide, mix fine with a small piece of stool with a "wooden stick," then close the slide and view the sample at 40X and 100X magnification[11]

"Floatation techniques"

Oocysts were isolated from feces by suspending 20-80 feces in PBS (pH 7.2) and centrifuging at 500 gm for 10 min. Dispose of the supernatant, enclose the pellet in a 15 ml centrifuge tube, add PBS (pH 7.2), 3-5 ml ether, and mix thoroughly. From bottom to top, separate the four layers by centrifugation at 500 g. One minute: (1-sediment,2-PBS,3-piece&4-solvent). The sediment layer, including 75% oocysts, decontaminates oocysts. This oocyst was prepared in 2.5percent $K_2Cr_2O_7$ before using [12]

Staining Procedure

- A sterile wooden stick with a cotton head was used to make a rectal fecal smear on a clean glass slide that had been let too dry at room temperature.
- Methanol was used to fix the dry smear for 5-10(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 10 seconds of tap water.
- 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.

The Ziehl–Neelsen method has been modified.

The "modified Ziehl-Nelseen stain M ZN-ST (acid-fast)" was used to examine rectal smears. Smearing was made permanent for 5 minutes with Methanol Alcohol and then left to dry naturally at room temperature. Carbol Fuchsin was used to stain the dehydrated smears for an hour, which was created by melting 15% "Carbol Fuchsin in methanol" before using it ("stock solution"). In 90 mL 5 percent phenol, add 10 mL Ziehl Fuchsin. Next, I washed my hands under running water. After that, agitate the slide for 20 seconds while diffusing a 2% H2SO4 solution. Tap water was used for rinsing. The smear was then stained for five minutes with a 5 percent malachite green solution, washed with tap water, and dehydrated. Then, 40X and 100X oil immersion objectives were used to examine the samples. [13]

Polymerase chain reaction multiplex (PCR)

"G. lamblia, E. histolytica, and Cryptosporidium." Detection of subunit ribosomal-based "rRNA genes" in human fecal samples using multiplex PCR. Follow these steps to perform this procedure:

Extraction of "Genomic DNA"

"Genomic DNA" from feces samples was extracted using "AccuPrep® stool DNA Extraction Kit" from "Bioneer Korea," following the manufacturer's instructions:-

- 200 mg "G. lamblia, E. histolytica and Cryptosporidium sp." Single positive stool samples were transferred to a sterile 1.5 ml micro-centrifuge tube, and then 20 L proteinase and 400 L stool lace buffer (SL) were added via Whirlpool.
- The tube was centrifuged at 12,000 rpm for five minutes after being incubated at 60 C for ten minutes.

• The supernatant was transferred to a newfangled tube, and each tube was given 200 l of binding buffer.

• Incubate the tubes at 60°C for another 10 minutes.

• < UNK> To remove any remaining liquid that had adhered to the tube's walls and lid, the samples were mixed through soft vortexing for 5second and then spun down for 10 seconds.

• The entire mixture (including any precipitate) was transferred to the "DNA filter column," which was housed in a 2-ml "collection tube." After that, the sample was spun at 8000 rpm for five minutes to remove any remaining debris. One of the flow-over tubes was ignored, and the column was put into a different 2ml collecting tube.

• The DNA filter column was loaded with 500 mL W1 buffer and centrifuged at 10000 rpm for 30 seconds. In order to restore the column to the two-ml collecting tube, the flow-through was removed and replaced with a fresh one.

• 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 tubes were centrifuged for one minute at 12000 rpm.

• A fresh 1.5 ml "microcentrifuge tube" was used to transfer the dried DNA filter column matrix, and 50 l of pre-heated "elution buffer" was added to the center of the column matrix.

• To ensure that the matrix absorbed the elution buffer, the tubes were let to stand for at least 5 minutes. The purified DNA was centrifuged for 30 seconds at 10000 rpm to elute it.

"DNA Genomic" Profile

A nanodrop spectrophotometer (THERMO, USA) was used to evaluate and measure the purity of "genomic DNA" isolated from feces samples (96samples) for all parasites, as follows:

• Select the relevant program after opening the nanodrop software (Nucleic acid, DNA). • The measurement pedestals were cleaned many times with a dry wipe. After that, pipetted 1 µl of H_2O onto the lesser measurement "pedestal's surface."

• The sample arm was lowered, the Nanodrop was initialized by clicking OK, and the pedestals and one l of the suitable were cleaned.

• Blanking solution was added as a black solution, which is the same elution buffer as the DNA samples.

• The pedestals are then cleaned, and one liter of DNA sample is pipetted for measurement. Pure DNA is retrieved when the absorbability rate is high enough (1.8).

Primers

Three PCR primers to detect "G.lamblia, E.histolytica, and Cryptosporidium sp." based on "ribosomal rRNA gene" were generated in the current study using "NCBI-Genbank (M54878.1, X64142.1, and AF112573.1", respectively) & 3 primer plus-design online. Bioneer, a Korean company, provided these primers, as shown in the table below:

PCR Size	Sequence	Primer	
574 bp	GTTGAAACGCCCG-TAGTTGG	F	*G.lamblia*
CTCGCTCGTTGTCGCAATG		R	
204 bp	ACGAGGAATT-	F	*E.histolytica*

	GGGGTTCGAC		
CACCAGACTTGCCCTCCAAT		R	
351 bp	AACCTGGTT-GATCCTGCCAG	F	*Cryptosporidium. sp*
TTCCCCGTTACCCGTCATTG		R	

Table 1: Primers, PCR, and sequence.

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
5 µ L		DNA template
1 µ L	GL- F	Forward primer (10pmol)
1 µ L	EH- F	
1 µ L	C.sp.- F	
1 µ L	GL- R	Reverse primer (10pmol)
1 µ L	EH- R	
1 µ L	C.sp.- R	
39 µ L		PCR water
50 µ L		Total volume

Table 2: Multiplex PCR master mix and the volume.

"PCR thermocycler" conditions using conventional "PCR thermocycler" system as shown in the following table

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

Table 3: PCR step.

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 applied to the agarose gel solution three times.

• It was necessary to place the gel solution into an empty tray, then wait for it to firm for 15 minutes at room temperature before gently taking the gel out from the tray and adding 10l of (100bp Ladder) and 5l of (100bp Ladder) to each comb well.

• 1X TBE buffer and the gel tray were introduced to the electrophoresis chamber. 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 shown (Samsung. china).

Results

The researchers examined the feces of 987 patients suffering from diarrhea. For "G.lamblia, E.histolytica, and Cryptosporidium sp.", they were examined under a light microscope using a direct wet mount method, and the DNA from the positive samples was extracted and sent to a "multiplex polymerase chain reaction (PCR)."

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 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 4: It used the direct smear technique, the infection percentage for parasites that cause "diarrhea" according to the residence area.

Gender	Ex-amind No.	In-fected No.	Parasites that cause diarrhea						Total per-centages 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 5: Diarrhea percentage of causing parasites by sex using the direct swab technique.

Age groups	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	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 6: Infection ratios of diarrhea-causing parasites by the direct smear method, broken down by age group.

Months	Ex-amind No.	In-fect-ed 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 7: Incidence of infection according to the collecting sample monthly.

Type of in-fection	Infected No.	%	Parasite type		
			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 8: Quantity and rates of parasite infection in Babylon province.

Residence area	Ex-amind No.	In-fected No.	Diarrhea-causing parasites						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 9: Using a multiplex PCR approach, the percentage of people infected with Diarrhea-causing parasites can be determined according to residence area.

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 10: Multiplex PCR incidence of studied parasite infection according to the patient's sex.

Age group	Examind No.	Infected No.	Diarrhea-causing parasites						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 11. Multiplex PCR incidence of studied parasite infection according to age groups of patients.

Months	Examind No.	Infected No.	Diarrhea-causing parasites						Overall percentages from 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 12. Multiplex PCR incidence of studied parasite infection according to the collecting sample monthly.

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
Duplex	22	66.7	*E.histolytica* + *G.lamblia*	22	66.7
Triplex	2	6.1	+ *E.histolytica* + *G.lamblia* *Cryptosporidium spp.*	2	6.1
Aggregate	33	43.4	-	33	43.4

Table 13: Types, quantities, and parasite infection rates in the Province of Babylon.

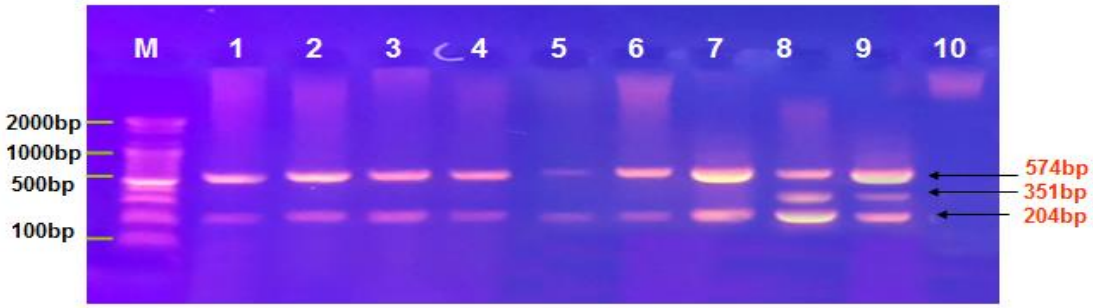

Figure 1: "Agarose gel electrophoresis image" showing the Multiplex PCR product analysis of the "ss rRNA gene" from "genomic DNA" from human fecal samples. Where M: Marker (2000-100bp), field (1-10) positive samples for "G.lamblia" at 574bp and "E.histolytica" at 204bp. Lane (8 -9) positive samples for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

Discussion

Direct smear testing for parasites that cause "diarrhea" can be used to determine infection prevalence in each area.

Babylon provincial residents were found to have a wide range of parasite infection rates by location, with the rural areas having a higher infection rate (67.2 percent) than those living in the city center (32.9 percent). The findings of [14] that Baghdad's rural population had this study corroborates the highest infection rates (50.9%). In Babylon province, infection rates increased 64.7% in rural regions while they declined 35.3% in urban areas, in agreement with [15]

Sixteen found that infection rates in rural areas were 79 percent higher than in the city areas, and infection rates in rural areas were 15 percent higher than in the city

areas [16,17] (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 most significant infection rates (45.3 percent), and the city had the lowest infection rates (25.9 percent). Study [17] indicated that the infection rate in rural areas was higher than in metropolitan areas (62.9 percent). This study supports this finding (37.9 percent). According to [18] in Al-Najaf, the rural area had a more significant infection rate than the metropolitan area (19.6 percent) (9.1 percent). Lack of clean water, direct dependence on river water as a source of water, lack of guidance and advice from authorities, poor health and cultural status of the rural population and lack of hospitals and health care in these centers. Biological use of animal waste and human waste are all factors that contribute to the high incidence of infections in rural areas—gender-specific prevalence of parasite infection in diarrhea patients as determined by direct smear cytology.

Males (51.5 percent) had the highest infection rates, and females (47.3 percent) had the lowest infection rates, with parasitic illnesses affecting 47.3 percent of both sexes (41.2 percent). According to a study by [14] in Baghdad, males have the highest rate of infection (58.5 percent), and females have the lowest rate of infection (48.5 percent) (41.5 percent), which agrees with Hilla's findings [19] as well. Males had the highest infection rate (53.9 percent) in his study, while females had the lowest (5.9 percent) (47.8 percent). As well as [20] in Baghdad, where he discovered the highest infection rates (15.35 percent) in females and (12.28 percent) in males, and [21] in Al-Sowera, where no significant differences between the sexes were detected, [22] disagrees as well—according to [16] males in the study areas (rural area and town) had 18.14% and 14.25% infection rates. In contrast, female infection rates were 18.14% and 14.25%, respectively (14.42 percent, 8.31 percent). Also, in Babylon's province, there was a quarrel with the [22]. The infection rate in men is the highest (80,6%), whereas the infection rate in women is the lowest (81 percent). The Al- Diwaniya province had the highest infection rate (6.12 percent) among men and the lowest infection rate (5.11 percent) among women, according to [23]

The prevalence of parasitic diseases was found to be 43.4% in this study, with males infected at the most significant rate (36.7 percent) and females infected at the lowest rate (0.1 percent) (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 [24] in Baghdad but contradicts [25], which found significant differences between men and women despite the highest infection rate among females (65.36 percent) and the lowest infection rate among males (14.58 percent) (60.13 percent).

Males' more significant activity and contact with the outside world and their status as a working group in the community may make them more relevant pathogens sick of women, as they eat and drink well in public places or at street vendors, as well as the anarchist nature of the movement and a general lack of attention to 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.

There was a wide range of age groups in which the prevalence of parasitic infections was measured [26] The infection rate was 76.1% for those aged 16-20, while the infection rate was 22.8% for those aged 26-30, followed by the 15-11 age group (61.9 percent) [[27] They agree with the study differed with [28], where he had the highest infection rate in the age groups (16-20) of 49.3%, followed by the age group (11-15) of 36.6 percent, in Baghdad (less than a year) When compared to

Baghdad, year one (the current study), [14] (39.5 percent). It was in Baghdad that he had the highest infection rate in the 15-11 age range, and it was also there that he disagreed with [20] that he had the highest infection rate in the 15-11 age range (15-11). (4-6). An analysis of parasite infection rates in people aged one month to more than thirty-one years was carried out using the PCR method. The age group (16-20) had the highest infection rate (46.2 percent), while the age group (16-20) had the lowest infection rate (16.7 percent). (21-25). Children under ten years of age in Baghdad had an infection incidence of 55.04 percent, according to this study. According to this study, the highest prevalence of diarrhea (29.6%) was found in the age groups of (12-23) months, followed by (15.6%) in 24-60 months, 8% in the 6-11 months, and 0% in the 0-5 months in Dar Es Salaam, Tanzania [29] (2.4 percent). Due to their lack of attention to personal hygiene and health conditions, students in the 20-16 age range are more likely to contract intestinal parasites than any other group. For cryptosporidium, the reason for the high incidence in this age range (less than one year) may be due to this parasite's opportunistic nature, which affects children and HIV-positive people. [26,27]

Conclusion

Based on the results, the following can be said: • Compared to previous studies, the number of parasites that cause diarrhea in the Babylon province is very high, and the infection rate is higher in rural areas than in cities. • There was a close link 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 five.

ACKNOWLEDGMENTS

Many thanks and gratitude to the "Department of Biology, College of Science for Women, University of Babylon" and laboratories and the "Al-Hilla Private University College, Department of Medical Laboratory Techniques" for conducting research and providing the required facilities.

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Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023
Citation: Al-Hamairy, A.K.; Mhdei, K.H.; Oleiwi, M.K. Prevalence Study of Major Protozoa Diarrheal Agents Among Patients in Babylon Province Using Microscopically and Molecular Methods. Revis Bionatura 2023;8 (3) 43. http://dx.doi.org/10.21931/RB/CSS/2023.08.03.43