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Molecular Epidemiological Characterization of *Giardia Lamblia* in Kirkuk, Iraq

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ABSTRACT: Results of laboratory examination of 600 faeces samples fem patients attended to Pediatric Hospital and General Kirkuk Hospital from January 2023 to December 2023 were showed 57 infection cases with *Giardia lamblia* with 9.5% infection rate. The diagnosis of positive samples was confirmed by PCR. No significant differences were observed in the prevalence of parasite in males 38.6% compared to 29.8% females. The highest infection rate 28.1% were found among age groups (1-5) years whereas the lowest infection rate 10.5% were found at age groups (11-15) years. The infection rate in rural area was 56.1% as compared to 12.3% in urban.

The PCR product analysis of glutamate dehydrogenase gene revealed two genotypes (assemblage A and assemblage B) amplified at 264 bp and 460 bp for identifying *G.lamblia*.

KEYWORDS: Molecular, Epidemiology, Giardia, Kirkuk, Iraq

I. INTRODUCTION

Giardia lamblia is a cosmopolitan parasitic protozoan flagellate that causes waterborne diarrheal disease in humans and several other vertebrates (Hajari, S. T., Chekol, Y., &Chauhan, N.2022) . In Iraq, it is considered to be one of the most common intestinal parasites, especially among children (Wielinga, C., Williams, A., Monis, P., &Thompson, R .2023). In addition to ingestion of contaminated water, the parasite may be transmitted by consuming foods (Mohmmed, B.A, Rasheed, Z .K, Jihad, L.J. & Abass, K.S. 2020).

Based on nucleotide sequences analysis of the small subunit ribosomal RNA (ssur RNA), glutamate dehydrogenase (gdh), B-giardin (bg) and triose phosphate isomerase (tpi), the G.lamblia complex has been grouped in to eight distinct assemblages (A-H) (Calegar, B C., Nunes, K. J. , Monteiro, P. A., Bacelar, B., & Evangelista, M. 2022). This includes human infection A-B, canids C-D, domestic animals E, cats F, rodents G and seals H (Zhang, J., Dan, L., Wang, H., Liu, Z., Zhou, X., Ma, Z., Ren, H., Fu, Y., .Geng, Y., Luo, Xie, G. & Peng, Z. 2021)

Since little attention has been paid to molecular and genetic diversity of G. lamblia in Iraq, this investigation was aimed to characterize the prevalence and genotype variation of strains isolated from human based on processing of amplified DNA with the glutamate dehydrogenase gene by using ordinary PCR.

II. MATERIALS AND METHODS

Collection of samples

A total of 600 human fecal specimens in pediatric hospital, Kirkuk, Iraq were collected between January 2023 and December 2023 Epidemiological data of patients concerning age, gender and place of residence were collected.

Staining and isolation of cysts

The fecal samples were smeared on to glass slide, stained with Lugol iodine stain and examined under light microscope.

The coprological analysis of the samples was carried out by centrifugation and flotation in 33% zinc sulfate with a density of 1-18 g/ml, in order to maximize the chance of finding Giardia cysts. The prevalence differences in relation to epidemiological data were tested by Pearson Chi- Square.

Extraction of DNA

DNA was extracted using (Stool DNA extraction package Favorge, Korea) according to the manufacturer's protocol. A frozen stool sample (20 mg) was solubilized by incubation with 30 ml of lysis buffer (pH 8.0, 10 mM EDTA, 10 mM Tris-HCl, 1 mg/ml K-protease) at 65 °C for 2 h followed by heating. Heat at 100°C for 30 minutes to denature the enzyme. Cell wreckage and proteins were removed by centrifugation (10,000 × g, 4°C, 10 min). The portion of the supernatant containing DNA was used for PCR, and 5 µl of the DNA solution was added to the PCR mixture. Assess the purity of DNA samples using optical density on a Nanodrop spectrophotometer. The purity of the DNA samples was assessed using an optical density of using a Nanodrop spectrophotometer (Hassan, H., Fadhil, M., &Fadhil, Z. 2016)

PCR Protocol

PCR amplification was performed using previously described forward (GCCATGCATGCCCGCTCACC) and reverse primers (GCGCTCCCGTTTCCTCGTGG), which were the previously reported to specifically amplify of the glutamate dehydrogenase gene of *G. lamblia*. The PCR reaction mixture (25µl) containing 10mM Tris-HCl, 0.2 mM each deoxynucleoside triphosphate, 25 pmol of each appropriate primer and 2.5 U of Taq DNA polymerase were prepared using a Perkin-Elmer thermal cycler with a thermal profile which composed of 32 cycles (preincubation at 94°C for 5 minutes, denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 1 minute and further incubation at 72°C for 10 minutes). Aliquots (10 µl) of the amplified PCR mixture was electrophoresed on a 1% agarose gel using X TBE running buffer (0.045 M Tris-borate and 1 mM EDTA).PCR product bands were visualized by ethidium bromide (0.2 mg/ml) staining (Bayraktar, M., Hussein, M., & Hassan, H. 2022).

III. RESULTS AND DISCUSSION

Direct microscopical examination of 600 stool samples in Pediatric and general Kirkuk hospitals revealed 57 positive samples with 9.5 % infection for *G. lamblia* (Table 1). These findings consistent with those in other provinces in Iraq and neighboring countries (Salman, Y., & Mustafa, M. 2013). The differences in the rate of infectivity may be due to many reasons such as immunity status, nutritional status, environmental factors and transmission routs [Hussein et al., 20090; Lazar et al., 2023; Barzinij et al., 2021; Ali, A. and Ali, Sh. 2020). The present investigation provide an alternative tool for molecular epidemiological diagnosis of Giardia species DNA directly in the stool samples of patients. Total DNA was extracted from all microscopically positive stool samples and used as templates PCR reaction. The gdh gene was amplified from 39 (68.4 %) samples and not amplified from 18 (31.6 %) samples. The negative PCR result in these stool samples may be due to the presence of other pathogenic Protozoan parasites.

The molecular characteristic of the isolates revealed implicating of a band of a size 264 bp and 468 bp (Figures1,2,3,4). which confirm the fact that the Giardia isolates could be classified in to assemblage A and assemblage B. This suggestion is supported by previous observations (Lee et al., 2006; Al-Asadi and Kadhum, 2018; Jeskea et al., 2022; Tokoro et al., 2023). Regarding to the residency, most infected patients with *G. lamblia* were living in the rural area with 56.1% infection followed by 12.3 % infection in urban area (Table 2). This study had obtained same results by others by which the rural area has the highest percentage of infection may be due to lack of clean drinking water availability and dependence of river water directly as a source of water as well as the use of animal waste and human feces as organic fertilizer for the growth of vegetables (Salim and Al- Aboody, 2019; AL-Saqur et al., 2016).

As shown in Table 3 the results revealed non-significant differences between gender and infectivity rate .The finding the infection rate of *Giardia* in male was of moderately higher in male (38.6%) than in female (29.8%) is in agreement with the results of (Hamza and AL-Ibrahimi, 2014; Mohammed, 2016 ; Hadi, 2014; Nujood, 2023). According to the age groups the highest infection rate of Giardia (28.1%) was within age groups (1-5) years followed by 15.8% infection in age groups (6-10) years, 14% in age groups under one year and 10.5%, age groups (11-15) years, which may be due to several factors, including defecation practices as these groups are independent in toilet use and have less awareness of hygiene rules such as washing hands before eating and after using toilet. Level of hygiene, socioeconomic status and poor health habits, are suggested to be risk factors for Giardia infection in children. In addition, the less mature Immune system in those < 6 years can reduce their ability to mount strong immune defense to infections agents [Tasawar et al., 2010; Al-Bayati et al., 2023; Salman and Ali, 2013; Salman, 2014; Ali and Hassan, 2021; Al-Ani and Al-Warid, 2023; Ahmed and Jasim, 2023).

IV. CONCLUSION

The main conclusion of the current study is confirming that the PCR technique is a sensitive method for diagnosing Giardia.

Table 1. Percentage of Infected and Non-infected Patients with *G.lamblia* by using PCR

PCR positive microscopy positive	Positive		Negative		Total	
	No.	%	No.	%	No.	No.
Giardia lamblia	39	68.42	18	31.579	57	100.00

Table 2. Distribution of the infected patients with *G.lamblia* according to Habitation by PCR

Habitation		No. of infected with *G.lamblia*	No. of non-infected with *G.lamblia*	Total
Urban		7	2	9
		12.3%	3.5%	15.8%
Rural		32	16	48
		56.1%	28.1%	84.2%
Total		39	18	57
		68.4%	31.6%	100.0%

P value 0.408

Table 3. Distribution of *G.lamblia* according to Gender by PCR

Gender			No. of positive sample	No. of negative sample	Total
	Male	22		8	30
		38.6%		14.0%	52.6%
	Female		17	10	27
			29.8%	17.5%	47.4%
Total			39	18	57
			68.4%	31.6%	100.0%

P value 0.400

Table 4. Distribution of the infected patient with *G.lamblia* according to age groups by PCR

Age group			No. of infected with *G.lamblia*	No. of non-infected with *G.lamblia*	Total
	<1	8		4	12
		14.0%		7.0%	21.1%
	1_5		16	4	20
			28.1%	7.0%	35.1%
	6_10		9	6	15
			15.8%	10.5%	26.3%
	11_15		6	4	10
			10.5%	7.0%	17.5%
Total			39	18	57
			68.4%	31.6%	100.0%

P value 0.556

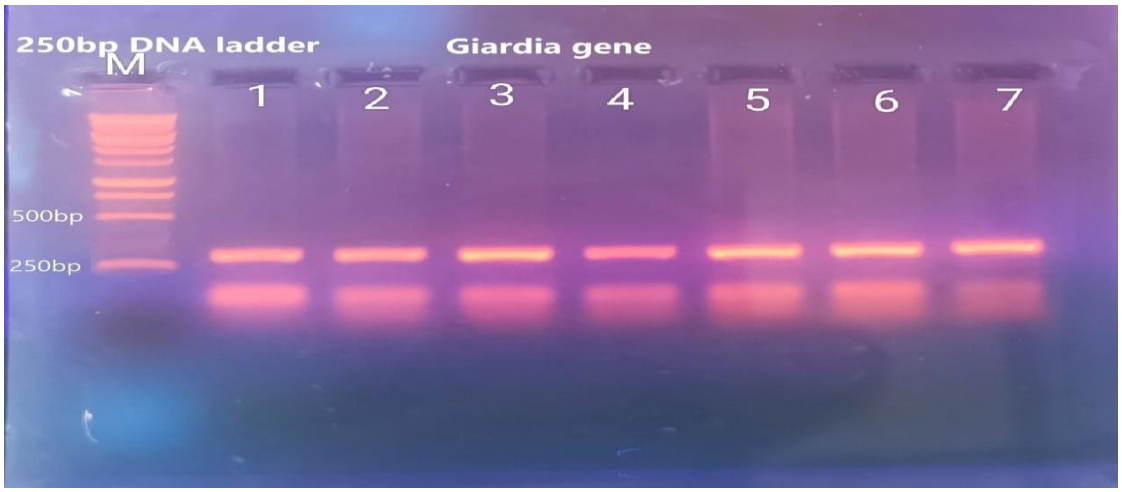

Figure 1 : Agarose gel electrophoresis analysis for PCR products stained with 0.2 mg/ml of ethidium bromide for glutamate dehydrogenases gene using primers species specific for *G.lamblia* obtained from human stool samples. Positive samples reveal 264 bp bands . M represent (100-2000)pb DNA Lader Marker.

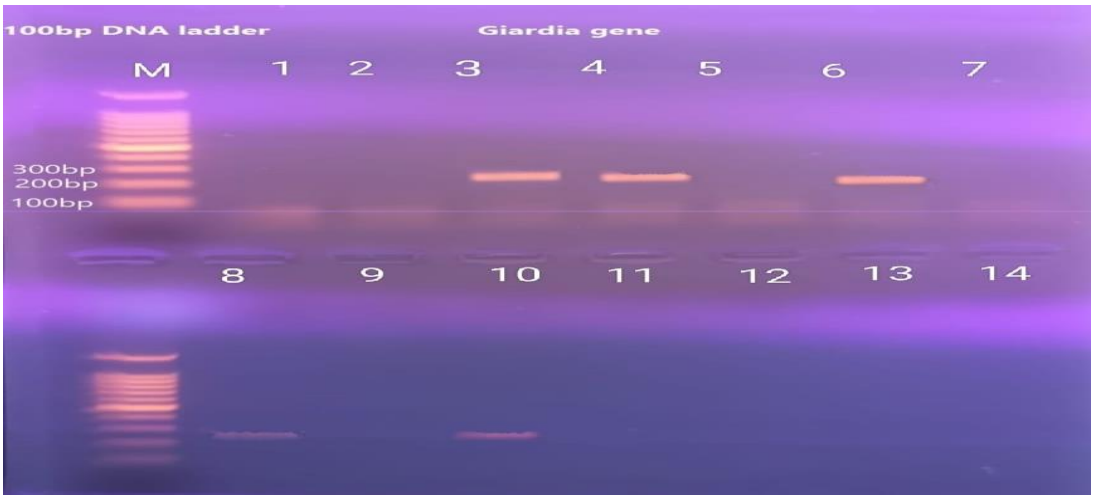

Figure 2 : Agarose gel electrophoresis analysis for PCR products stained with 0.2 mg/ml ethidium bromide for glutamate dehydrogenases gene using primers species specific for *G.lamblia* obtained from human stool samples. Positive samples reveal 264 bp bands . M represent (100-2000) pb DNA Lader Marker.

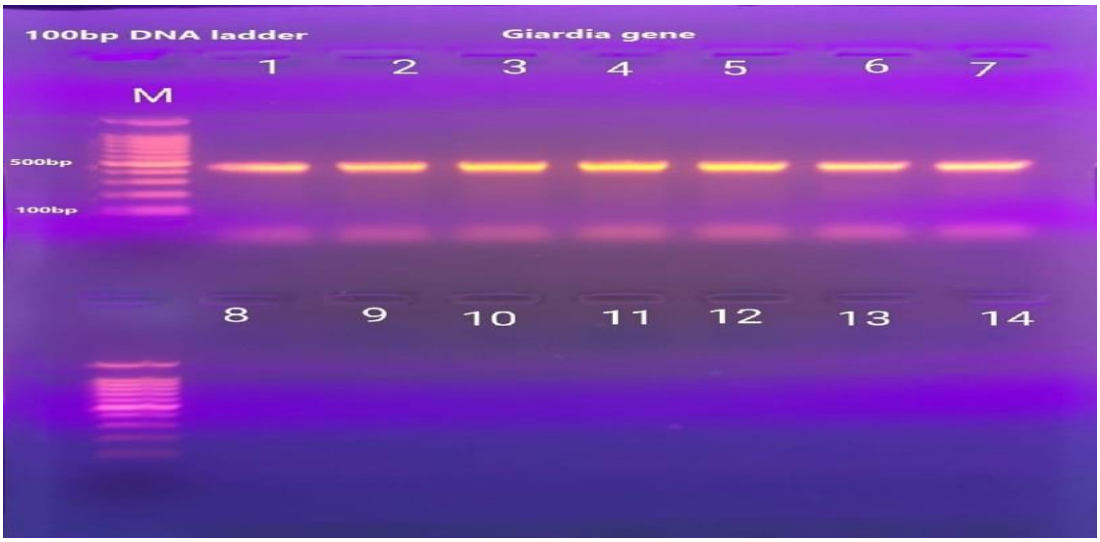

Figure 3 : Agarose gel electrophoresis analysis for PCR products stained with 0.2 mg/ml ethidium bromide for glutamate dehydrogenases gene using primers species specific for *G.lamblia* obtained from human stool samples. Positive samples reveal 460 bp bands. M represent (100-2000) pb DNA Lader Marker.

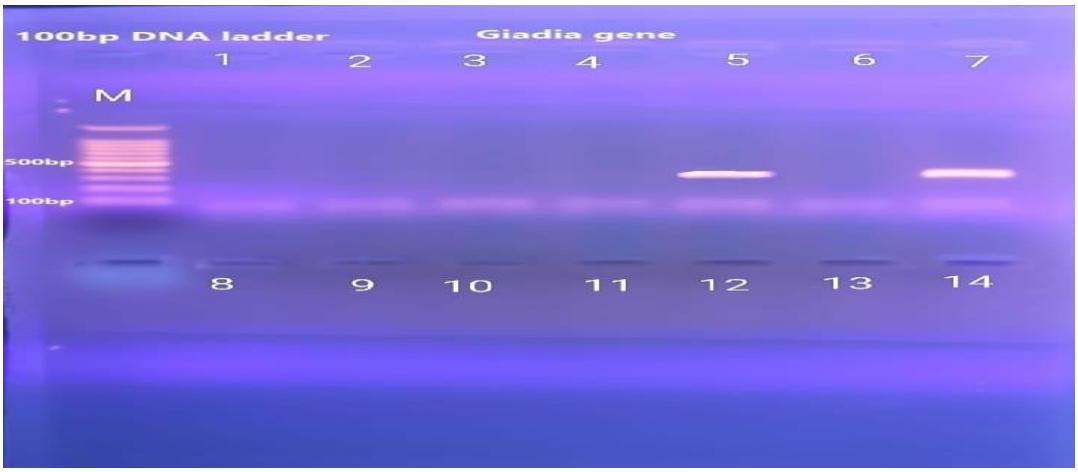

Figure 4 : Agarose gel electrophoresis analysis for PCR products stained with 0.2 mg/ml of ethidium bromide for glutamate dehydrogenases gene using primers species specific for *G.lamblia* obtained from human stool samples. Positive samples reveal 460 bp bands. M represent (100-2000) pb DNA Lader Marker

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