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Microscopic and Molecular Diagnosis of *Giardia Duodenalis* in Human in Babylon Province, Iraq

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ABSTRACT

Considering the importance of *G. duodenalis* to public health and the country's economic situation due to the threat of an outbreak and economic losses . Thus, this study aimed to detect *G. duodenalis* in human in Babylon province using traditional and molecular techniques. This study was conducted in Babelon province from 1st of Oct 2022 to 30th of March 2023. A total of 100 human stool samples were examined microscopically for detecion of *G. duodenalis* from both diarrheal and non diarrheal patients attend hospitals and public health centers laboratories . By using microscopic examination. The rate of infection with *G.duodenalis* was 34/100 (34%). According to sex, the highest infection rate in humans was in males 19/51(37.25%) compared to females 15/49 (30,61%). Significant differences (P≤0.05) was recorded in the rate of infection between age groups, the highest rate 17/29 (58.62%) observed in (5-14 years) of human. Rural area revealed the highest rate of infection than urban area in human 24/55 (43.63%) with significant differences (P≤0.05). The highest infection rate in humans 10/17 (58.82%) was in November with no statistically significant differences (P≤0.05). In molecular diagnosis, DNA extraction was performed for 40 stool samples human, in order to investigate the genotypes of *G. duodenalis* from human in Babylon, seven *Giardia* isolates. were genetically characterized by *SSU-rDNA* gene sequencing. The results showed the percent of assemblage A was 5/7 (71.4%), while assemblage B was 2/7 (28.6%). These findings suggested that infection of humans by zoonotic genotypes A and B were the most common genotypes detected in all human samples in Babylon province, demonstrating the importance of genotyping identification in understanding the transmission routes and epidemiology of giardiasis.

KEYWORDS: Giardia duodenalis; human; Microscopic ; molecular diagnosis.

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INTRODUCTION

Giardia duodenalis is a protozoan parasite that infects humans in addition to a wide range of domestic and wild animals. In Asia, Africa, and Latin America, around 200 million individuals suffer from symptomatic giardiasis, with over 500,000 new cases reported yearly [1]. Since 1971, there have been over 300 waterborne giardiasis outbreaks documented globally [2, 3]. Many individuals with *G. duodenalis* in stool samples are asymptomatic. However, *G. duodenalis* has been found in the stool of asymptomatic people more frequently than in those with acute diarrhea [4]. Giardiasis is highly prevalent because of various causes, including the environment, unsanitary conditions, and contaminated drinking water [5]. According to genetic research on a few genetic markers, *G. duodenalis* is classified as a species complex made up of eight genetic assemblages (A–H). Because they have been found in animals as well as humans, Assemblages A and B are known to be harmful to people and are thought to have zoonotic potential [6]. Other assemblages, such as assemblages C and D in dogs and assemblage E infects domestic ruminants and pigs, assemblage F infects cats, and assemblages G and H infect rats and marine mammals, respectively [7, 8]. Giardiasis can be diagnosed in the lab using fecal microscopic examination , a variety of immunological-based tests and molecular approaches. Polymerase chain reaction (PCR) has been shown to be more accurate and useful than the Enzyme Linked Immunosorbent Assay (ELISA) and fecal microscopy for diagnosing *G. duodenalis* infection [9]. Considering the

importance of *G. duodenalis* to public health and the country's economic situation due to the threat of an outbreak and economic losses . Thus, the aim of this study is to detect *G. duodenalis* in human in Babylon province using microscopic and molecular techniques. To genotype the isolates by *SSU-rDNA* gene sequence analysis.

MATERIALS AND METHODS

This study was conducted in Babylon province from 1st of Oct 2022 to 30th of March 2023. A total of 100 human stool samples were examined microscopically for detecion trophozoites and/or cysts of *G. duodenalis* from both diarrheal and non diarrheal patients attend hospitals and public health centers laboratories. During the collection of fecal sample color and consistency were recorded for each hosts.

DIRECT WET SMEAR

A small amount of feces was taken with a pin stick, mixed well on the glass slide, and covered with the cover slide before it was examined under the microscope at (40x and 100x) magnifications. The stained smears with Lugol's iodine were prepared by taking a few amount of feces mixing with a with drop of Lugol's iodine stain on the glass slide [10, 11].

Concentration methods:

Flotation technique

Fecal floatation based on zinc sulfate was done by using 2 to 4g of feces. The feces and zinc sulfate solution (specific gravity 1.18) were centrifuged with a cover slide in place for 5 minutes at 200xg. After centrifugation, the coverslip was removed to a glass slide and scanned for *G. duodenalis* trophozoites and/ or cysts with light microscope at 40x and 100x [12].

Cysts measurement:

Cysts were measured by using Leica microscope with digital camera (scope image 9.0/ China) which was provided with image processing software. The software of camera was calibrated to all lenses of Microscope-leica by aid of 0.01mm stage micrometer (ESM-11 / Japan).

Molecular study

DNA extraction and PCR analysis

DNA was extracted directly from feces according to manfucture instructions using (*EasyPure*®Stool Genomic DNA Kit, Transgen company/China).Conventional PCR was preformed for the detection and identification DNA of *G. duodenalis* of 40 sample, including microscopically positive samples for the purpose of confirmation.. A 292 bp region of the 5' end of the *(SSU-rDNA*) gene was amplified using primers RH11(F) 5'-CATCCGGTCGATCCTGCC-3 and RH4(R) 5'-AGTCGAACCCTGATTCTCCGCCCAGG -3, as described by Hopkins [13]. Denaturing step at 96 ◦C for 3 min, followed by 35 cycles of denaturing for 45 s at 96 ◦C, annealing for 30 s at 50 ◦C and extension for 45 s at 72 ◦C, followed by a final extension at 72 ◦C for 7 min.

DNA sequencing:

DNA sequencing analysis was carried out for identification of *G duodinalis*, seven PCR positive of local isolate products were sent to Macrogene company to identify *G. duodenalis* genotypes. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center (http://www.ncbi.nlm.nih.gov), and BioEdit program. The results were compared with data obtained from Gene Bank published ExPASY program which is available at the NCBI online.

Phylogentic Tree:

The evolutionary distances were computed by phylogenetic tree Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method [14].

Statistical analysis:

Statistical analysis was carried out using the Statistical Package for Social Science (SPSS) version 27 for window software and Microsoft Excel 2010 [15].

RESULTS

Microscopic examination:

These results also demonstrated oval shape of *G. duodenalis* cysts contains (4) nuclei, axostyl in central cyst and thick wall with brown color when use iodine stain, average size 12.5 × 8.5 ± 1.5µm (Figure 1). Also pear-shaped mobile trophozoites which contain four pairs of flagella and two spherical nuclei. T

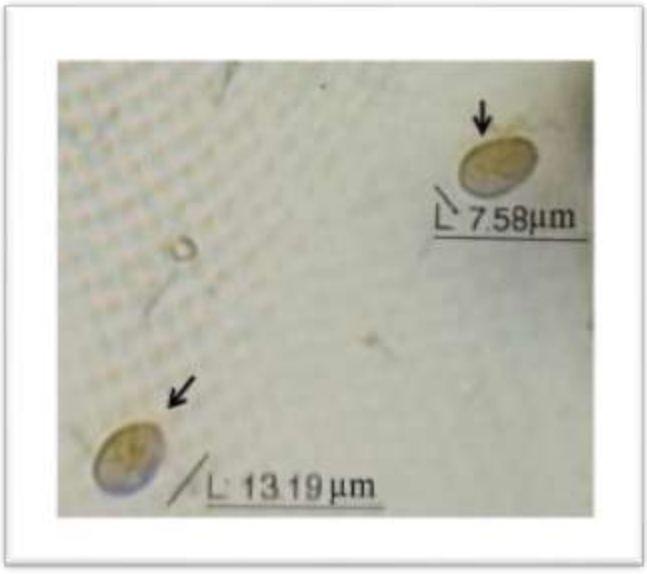

Figure 1: *G. duodenalis* cysts (as shown in black arrow) isolated from human stool samples by Zinc Sulfate Flotation Technique with iodine stain : 40X.

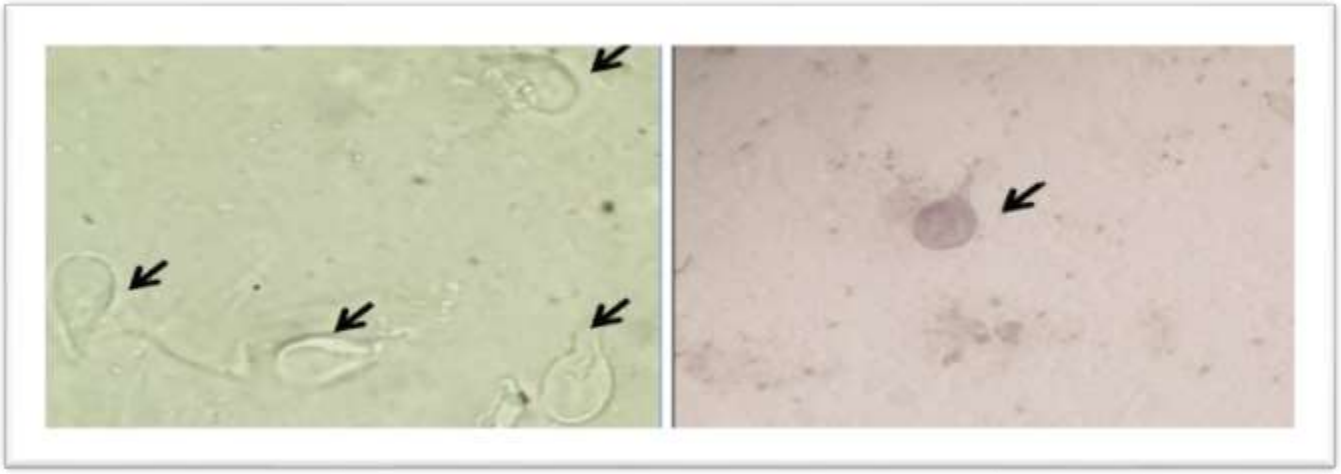

Figure 2: *G. duodenalis* trophozoit (as shown in black arrow) in human stool samples (right) direct smear method (left) stained with Geimza stain: 40X.

By using microscopic examination. The rate of infection with *G.duodenalis* was 34/100 (34%). According to sex, the highest infection rate in humans was in males 19/51(37.25%) compared to females 15/49 (30,61%). Significant differences (P≤0.05) was recorded in the rate of infection between age groups, the highest rate 17/29 (58.62%) observed in (5-14 years) of human. Rural area revealed the highest rate of infection than urban area in human 24/55 (43.63%) with significant differences (P≤0.05). The highest infection rate in humans 10/17 (58.82%) was in November with no statistically significant differences (P≤0.05). (Table 1).

S: significant differences (P≤0.05), NS: No significant differences (P≤0.05).

Table 1: Infection rates of G. duodenalis in human according to epidemiological featutes

Features		Examined samples	Infected samples	Infection rate %	P-value	X^2
Sex	Male	51	19	37.25%	0.491 NS	0.483
	Female	49	15	30.61%		
Age groups	< 5y	16	5	31.25%		
	5-14 year	29	17	58.62%		
	15-25 year	22	6	27.27%	0.02 S	12.472
	26 -45 year	12	2	16.66%		
	46-60 year	12	3	25%		
	> 60 year	9	1	11.11%		
Area	Rural	55	24	43.63%	0.025 S	5.058
	Urban	45	10	22.22%		
Months	Oct.	17	7	41.17%		
	Nov.	17	10	58.82%		
	Dec.	17	5	29.41%	0.089 NS	9.51
	Jan.	17	3	17.64%		
	Feb.	15	6	40%		
	Mar.	17	3	17.64%		

Molecular study

Conventional PCR

DNA extraction was performed for 40 human stool samples, including microscopically positive samples, for the purpose of confirmation. The results showed that the PCR amplification of the *SSUrDNA* gene was positive for 37samples (Figure 3).

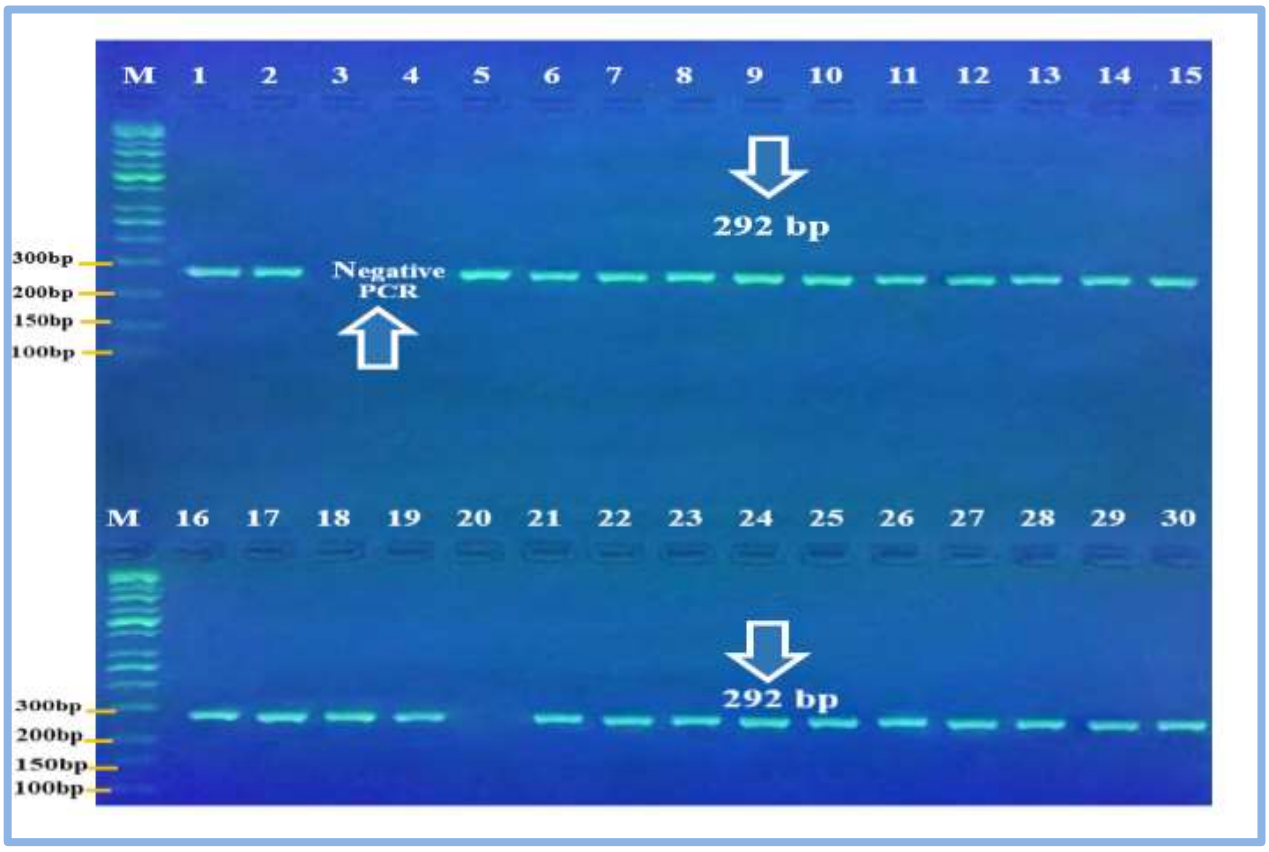

Figure 3: PCR product of *G. duodenalis* , the band size (292bp). 1.5% agarose.TBE 1x buffer for 1:30 hours. M: DNA ladder (100).

Sequence analysis of *G. duodenalis* :

After confirming the amplification of gene by conventional PCR, seven PCR-positive samples of local isolates products were sent to Macrogene company to identify *G. duodenalis* genotypes. All seven PCR-positive samples were successfully sequenced, among them, 5 reported for Assemblage A (71.4%), 2 for Assemblage B (28.6%) (Figure-4). *G. duodenalis* isolates were submitted in NCBI genbank data base and genbank accession numbers were obtained. Sequences of isolates alignment with references isolates for *G. duodenalis* which previously recorded in genbank. Seven *G. duodenalis* isolates with accession numbers OR072990.1, OR072991.1, OR072992.1, OR072993.1, OR072994.1, OR072995.1, OR072996.1 were showed closed related to NCBI blast *G. duodenalis* of Uganda.

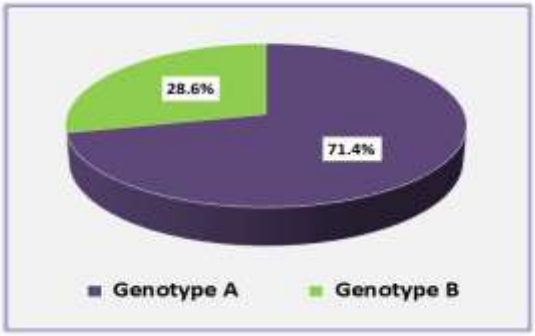

Figure 4: Genotypes of *G. duodenalis* in human

Phylogenetic Tree:

The evolutionary history was inferred using the UPGMA method. The optimal tree with the sum of branch length = 0.02117480 is shown (Figure 5).

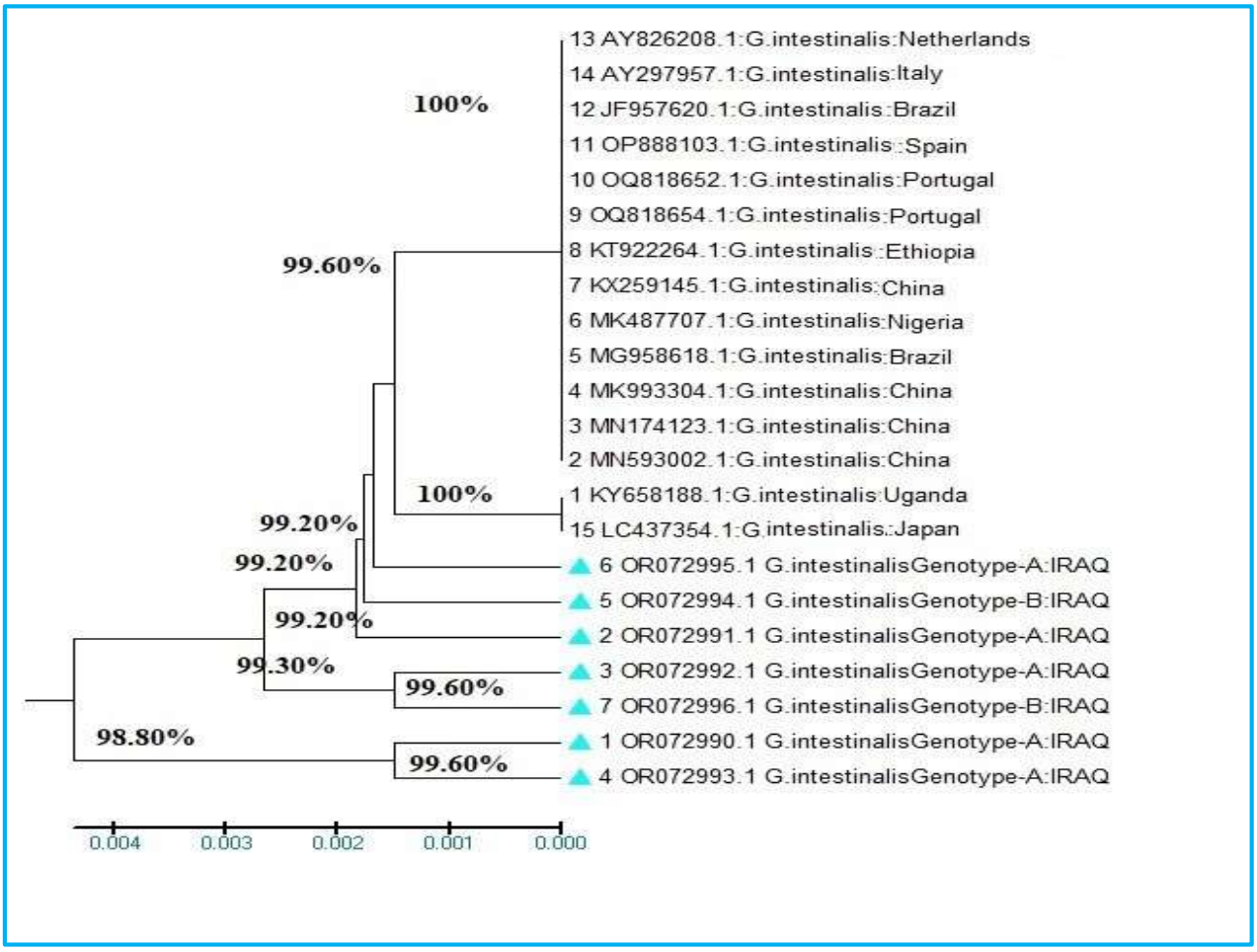

Figure 5: Phylogenetic tree analysis based on *SSUrDNA* gene partial sequence explains genetic identity between the local ioslates of *G. duodenalis* in human and NCBI-BLAST isolates.

DISSCUSION

In this study, microscopic and molecular diagnosis was applied to identify *G. duodenalis* from fecal samples in human in Babylon province. *G. duodenalis* diagnosis is based on microscopic examination; this technique is unable to differentiate between the genetic assemblages of *G. duodenalis* isolates. Thus, the molecular technique has been developed for genotyping identification of *G. duodenalis* [9, 16]. By using microscopic examination. The rate of infection with *G. duodenalis* was 34% in human. This result was in agreement with the results of a study conducted in Hillah, where the infection rate in human was (31.3%) [17], as well as it agreed with results of Latif, Al-Talib [18] in Baghdad, where it was recorded (33.33%) infection rate of *G. duodenalis* in human. Poor socioeconomic condition, low standard of sanitation and hygiene and lack of education contributed to the high infection rate of *G. duodenalis* . [19, 20]. Comparatively infection rate of both sexes in human, the rate of infection was in the male higher than rate of infection in females. These findings agreed with another study in Babylon, where males had higher infection rate compared with females [21]. However, this result contradicted the findings of Al-Sherefy and Al-Hamairy [17] in Hillah, where they found the highest rate of infection in females. The difference in infection rates between males and females might be ascribed to the fact that males are the most movement and active and their contact with the external environment factors, as well as males eat and drink in public places or from street sellers, this is what makes them more relevant pathogens than females [17]. In current study, significant differences ($P \leq 0.05$) was recorded in the rate of infection between age groups, the highest rate observed in (5-14 years) of human. These results were consistent with a study included all provinces of Iraq during 2015, where the majority of cases were recorded among (5-14) groups [22]. In addition, it agreed with other previous studies [5, 23]. This result, however, disagreed with the findings of Ali, Mero [24] in Duhok province/Kurdistan Region, where the highest rate of infection was observed in >3-6 years age group. The high rates of infection with *G. duodenalis* in age groups (5–14years) might be due to this group's ignorance and lack of knowledge of general hygiene rules, as well as the fact that

children at this age are more mobile and active, as well as children's habits of playing in contaminated soil in pools or sewers with stagnant water [25, 26], perhaps the cause is immunodeficiency of these children [27]. Rural area revealed the highest rate of infection than urban area with significant differences (P≤0.05). High infection rates in rural areas may due to several factors including; lack of clean drinking water and dependence on river water as a direct source of water, dealing with the contaminated soils of gardens and farms with parasite cysts, breeding and contact with animals that are reservoir of the parasite, using animal waste as organic fertilizer, and the low health and cultural levels of the rural population [28]. In the current study *G. duodenalis* infection was increasing during November (58.82%), followed by October (41.17%). The lowest infection was in January (17.64%), which was consistent with the findings of Hasan, Muhaimid [28], recording the lowest rate of infection in January (6%). However, the current findings disagreed with Khudhair [29] who recorded the highest rate of infection in July (4.9%) and lowest rate was observed in November (3.2%) among residents of Hawler, Soran and Chamchamal Cities, North of Iraq. While Shahatha [30] recorded the highest rate of infection was 62.5% during June, while October was the lowest rate 5%. Seasonal variations in infection rates were hypothesized to be caused by changes in temperature, rainfall, and moisture, which may facilitate infection of water-borne diseases, which may become significant in certain areas if climate change continues to cause a shortage of clean surface water [31]. In regards to the molecular results in human, PCR was used to amplify the genes encoding the *SSU-rDNA*. The conserved *SSU-rDNA* gene is a commonly used marker for assemblage differentiation (mostly genotyping) of *G. duodenalis* [6, 32]. In human, the percent of assemblage A was higher than assemblage B in Babylon city, this result was in agreement with study in Wasit where the perecent of genotype A was higher than genotype B [33]. Also similar to other study in Australia [34]. In contrast, the result of another study in Al-Diwaniyah province, which noted that the genotype B is the most common than genotype A [35]. *G. duodenalis*, Assemblage A is the prevailing genotype in the current study. This assemblage is primarily linked with zoonotic transmission, while assemblage B is more coupled with human-to-human transmission [36]. According to the phylogenetic tree, the isolates of *G. duodenalis* in the current study had similarity to sequences deposited in GenBank, with the maximum homology of the *SSUrDNA* gene sequences between the Iraq *G. duodenalis* isolated strain and world strains being (98.8% -100%). Phylogenetic analysis confirmed low differences between Iraqi strains of *G. duodenalis* and other countries. This genetic variation may be due to differences in area size of the reference sequence and differences in geographical areas where isolates collected; For genetic analysis, different methods such as PCR based on gene sequencing of partial or complete genes were used. The results of this study agreed with some studies that reported the existence of genetic variation and phylogenetic relationships among parasite populations that analyze genetic variations of *G. duodenalis* around the world [37].

CONCLUSIONS

The current study indicates that the frequency of *G. duodenalis* infection in human in rural areas of Babylon province is higher than urban areas of iraq. Children (5-14 years) recorded the highest infection rate compared to other age groups.findings suggest that infection of human by zoonotic genotypes (A&B) could be epidemiological significance. It is critical to provide clean and safe drinking water, proper sanitation, and health education about personal hygiene practices, particularly hand washing, as well as identifying and treating infected family members, and these interventions should be included in a strategy to control intestinal parasites among these communities in order to reduce *G. duodenalis* transmission and morbidity.

Ethics approval

The current research has followed the accepted principles of ethical conduct by College of Veterinary Medicine / Al-Qasim Green University (No: 2022 , 11/10/2022).

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