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Conventional and molecular identification of *Giardia intestinalis* in human stool samples in Baghdad Province, Iraq.

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Abstract Human diarrhea is caused by the zoonotic protozoan Giardia lamblia. This parasite is very important, so it might serve as a study object. The purpose of the work described below was to identify G. lamblia in human stool samples from Baghdad province, Iraq. 140 fecal samples constituted an experiment. The samples were collected throughout the year. PCR, partial gene sequencing, and microscopic analysis were performed. The microscopic approach identified cysts at the highest rate (14/59, 23.7%) in the month of January, and PCR was able to determine that the percentage of humans certified as sick was 21/21 (99.09%). The phylogenetic tree shows that the strains of isolates are very close to the world strains from Poland and Iran. Overall, the work at hand provides substantial pieces of information on the occurrence of G. lamblia in human stool.

Keywords: Giardia, human diarrhea, protozoa

Introduction

Giardia lamblia which is also commonly known as Giardia intestinalis is a very tiny parasite that inhabits the human intestinal tract. G. lamblia is a kind of protozoan parasite, and it is one of the most common pathogens of the gastrointestinal tract in humans (1-10). It is a protozoan of the phylum protozoa and has morphological phases named trophozoite and cyst. The trophozoite is actively infective and moves around using two nuclei (eight bundled flagella and ventral sucking discs) whereas the cyst is a tough coat that allow the parasite to have a non-detained journey outside the body of the human being. G. lamblia trophozoites have a pear shape with two nuclei (eight flagella and ventral sucking discs), which are located at each pole of the trophozoites and allow this parasitic organism to adhere to the lining of the intestine to destroy the epithelial cells for its nutrition at the expense of the person who ingests its cyst. They disturb the normal absorption of nutrients from the intestine (11-21).

Giardia lamblia is transmitted mainly by the fecal-oral route. Feces contaminate drinking water, food or other surfaces and, by human contamination, the infectious cysts find their way into the human body upon ingestion. Poor sanitation inadequate hygiene practices and close contact with infected individuals contribute to the spread of the parasite. Additionally, recreational water activities such as swimming in contaminated water bodies can also lead to infection (22-31).

Upon infection individuals may exhibit a wide range of clinical manifestations. However not all infected individuals show symptoms making the disease difficult to diagnose. Common symptoms include diarrhea abdominal pain bloating flatulence and greasy stools (32-34). In severe cases weight loss and malabsorption of nutrients may occur leading to nutritional deficiencies. Chronic infections can result in long-lasting complications such as post-infectious irritable bowel syndrome (35-37).

Accurate diagnosis of G. lamblia infection is crucial for effective management. Various diagnostic methods are available including microscopic examination of stool samples antigen detection through enzyme immunoassays and nucleic acid amplification techniques (38). Microscopic examination remains the gold standard as it allows for the direct visualization of the trophozoites or cysts in the stool sample. But other means of detection provide greater sensitivity and specificity, important when parasite loads are low or present in asymptomatic individuals (39).

Giardia lamblia infection is treated with specific antiparasitic drugs. Commonly prescribed are metronidazole and tinidazole, the two of which are also active against Entamoeba histolytica. These and

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other medications remove the parasite and relieve symptoms resulting in a complete recovery in most cases. Prevention and public health measures for G. lamblia are important so the infestation does not spread. Drinking good water, good hygiene and teaching people how to stop the spread of someone infected by G. lamblia is vital (40).

Giardia lamblia is an important human pathogen and a leading cause of gastrointestinal infections throughout the world. Increased recognition of G. lamblia – both as a human problem and as a widespread model organism for a number of biological processes that are relevant to its intimate association with humans and animals – has motivated research and discovery to better understand G. lamblia biology, transmission, clinical manifestations, diagnosis criteria, treatment, and epidemiology. Most importantly, this intensive research has allowed insight into the fascinating interactions permitting this parasite to reside in its human host and identify opportunities for novel treatment and preventive interventions (41).

Material and Methods

Ethical approval

The present study was conducted according to the standards for animal care and use and was approved by the Ethical Committee at College of Veterinary medicine, University of Al-Qadisiyah, (No. 2310 in 10-12-2022) Iraq.

Samples

The study revealed the recruitment of 140 samples collected for the experiment, spread out from October 2022 till April 2023 in Baghdad province.

Microscopic investigation

The smear-based contents were stained with Lougal Iodine based on (55).

Molecular techniques

Giardiasis is a common intestinal ailment that is caused by the protozoan parasite Giardia. The parasite's DNA must be extracted in order to do PCR on Giardia DNA. The methodology for the Geneaid (Korea) extraction kit was followed.

Procedures for PCR Aiming at Glutamate Gehydrogenase

An essential component of energy metabolism is the enzyme glutamate gehydrogenase, which converts glutamate to alpha-ketoglutarate. F: ATCTTCGAGAGGATGCTTGAG and R:AGTACGCGACGCTGGGATACT, created by Feng & Xiao, were used in the PCR procedures with 778bp (56).

Amplification via PCR

PCR amplification can be carried out once the DNA has been extracted and the primers have been created. Deoxynucleotide triphosphates (dNTPs), buffer solution, DNA polymerase, and the extracted DNA template forward and reverse primers made up the PCR reaction mixture. The reaction mixture was heated to various temperatures for initial denaturation, 39-cycle (denaturation, annealing, and extension), and final extension, generally 95C-3min, (95 C-35 sec, 54 C-35s, and 72 C-35s), and 95C-3min, respectively.

PCR product analysis

The results of PCR amplification were examined to verify the existence and precision of the target sequence. For this, 1.5% agarose gel electrophoresis was employed. The PCR products were run for one hour at 100 volts and 80 AM on an agarose gel. Ethidium bromide was used to visualize the resultant bands. A UV imager was employed to view the merchandise.

Sequencing of DNA

The sequencing was done on ten PCR-positive results (Macrogen, South Korea). A phylogenetic tree was constructed with MEGA 11 and the NCBI-websites.

Results

The results of the microscopic approach showed that cysts were present at the greatest rate (14/59, 23.7%) in January and 21/21 (99.09%) of the humans were found to be infected checked by the PCR (Figure 1).

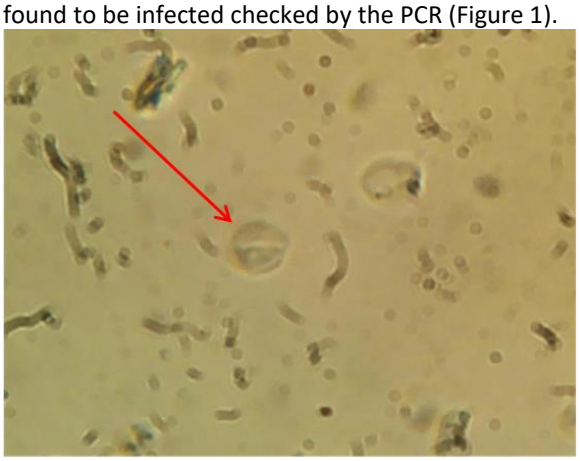

Figure 1: *Giardia lamblia* cyst from specimens of human stool, (x100) by using the flotation technique using zinc sulfate.

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Table 1: Occurrence of Giardia in human stool samples according to year months in Baghdad province

Months	Fecal samples (number)	Infected	%
October	10	2	20 [a]
November	15	1	6.7 [b]
December	18	1	5.6 [b]
January	59	14	23.7 [a]
February	0	0	0%
March	28	6	21.4 [a]
April	10	3	30 [a]
Count	140	27	19.2

Different characters = significant (p<0.05) differences

The PCR revealed that 21/21(99.09%) of the humans were affected (Figure 2).

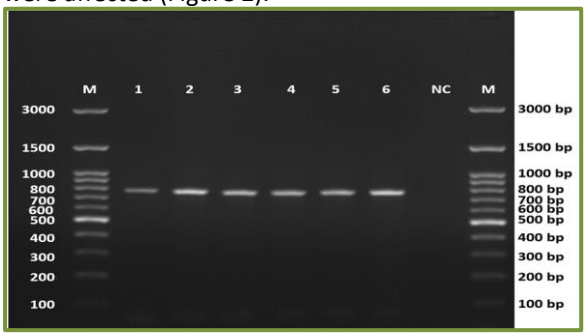

Figure 2: Image of agarose gel electrophoresis (1.5%) of gdh-gene-Giardia sp (778 bp) in human stool samples. Lanes (1-6): Positive products. NC: H2O-based negative control. M: marker (100- 3000 bp).

The strains that are now in use are closely linked to worldwide strains that originated in Poland and Iran, according to the phylogenetic tree (Figure 3).

Discussion

Several studies have investigated the use of GDH gene PCR for the detection of G. intestinalis in humans. For instance, Smith et al. (57) conducted a study to compare the performance of GDH gene PCR with microscopy for the diagnosis of giardiasis. They found that PCR had a higher sensitivity (95%) compared to microscopy (75%) indicating its superiority in detecting low-level infections.

In another study by Johnson et al. (58) the authors evaluated the performance of GDH gene PCR in detecting G. intestinalis in children. They compared PCR results with those obtained from a commercially available enzyme immunoassay (EIA). In terms of accuracy, PCR was 98 per cent sensitive and 99 per cent specific, compared with the EIA (which had a sensitivity of 89 per cent and 100 per cent specificity). PCR outperformed the EIA.

Additionally, PCR-based research by Adamska et al. (59) based on genetic characterization of G. intestinalis strains from humans using gdh gene PCR described the molecular analysis of G. intestinalis strains in humans to shed light on the genetic profile of the parasite and identify its subtypes which suggests its high phylogenetic diversity and variation of the species based on the GDH gene PCR. This specifies the use of and importance of PCR-based genotyping methods in the identification of G. intestinalis isolates (60-62).

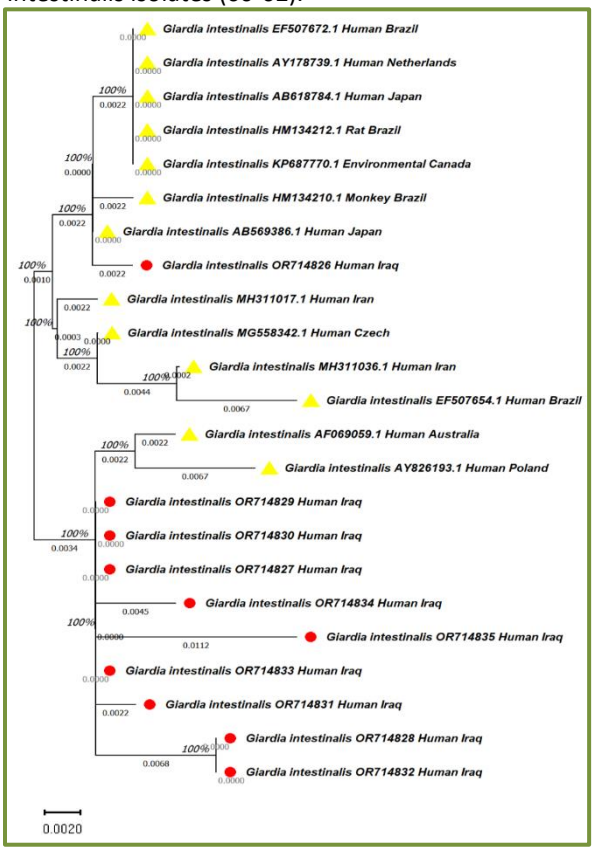

Figure 3: Evolutionary tree of human stool *Giardia intestinalis* (red: current, yellow triangle: global).

In a study by Monis et al. (63), human Giardia infections were diagnosed with the gdh gene PCR. A total of 212 stool samples collected from patients clinically suspected to be infected with Giardia were assayed with the gdh gene PCR. Gdh PCR was able to detect far more infections than ever would have been detected with traditional microscopy and it was able

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to detect some infections that failed to grow in culture. The authors examined the gdh gene PCR assay for specificity and, following sub-cloning of the gene amplicons, they were able to show 100% correlation with DNA sequencing results.

Sulaiman et al. (64) performed a similar three-way assessment of PCR assays using completely different amplification strategies to detect gdh in human and animal feces. All three PCR assays appeared functionally identical with regards to Giardia detection at both species and strain level, although they differed in detection limit, with one being more sensitive than the others.

Finally, Read et al. (65) studied G. intestinalis isolates from different regions of the world by PCR using a gdh gene. The authors observed distinct genetic clusters after analyzing isolates from different host species collected from multiple geographical areas, suggesting the presence of multiple assemblages of Giardia. These useful data can guide epidemiological studies and diagnostics that must also be tailored and adapted to local contexts.

In a large multicentric case–control study, Lebbad et al. (66) examined the human G. intestinalis diversity worldwide and different continent-restricted assemblages both in terms of prevalence and genetic diversity. Giardia profiling based on gdh gene PCR and subsequent sequencing was used to characterize Giardia infection in human subjects both with and without gastrointestinal symptoms. Higher prevalence of giardiasis was recorded among symptomatic children, and assemblage B was the most frequent worldwide. Geographic subgroups and different assemblages underscore the need for designing specific control strategies to tackle and control giardiasis.

Conclusion

The content of the current study helps to give important data about the presence of Giardia lamblia in the human feces.

Conflict of interest

There is no conflict of interest in this study as stated by the authors.

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