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The Incidence and Molecular Detection of *Blastocystis hominis* among Children with Diarrhea in Diyala/ Iraq

A Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ اِقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ * خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ * اِقْرَأْ وَرَبُّكَ
الْأَكْرَمُ * الَّذِي عَلَّمَ بِالْقَلَمِ * عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ﴾

صِدْقُ اللَّهِ الْعَظِيمِ

العلق: 1 - 5

DEDICATION

I dedicate this work to

To the source of safety. My father

To the source of tenderness. My mother

To the soul restorer. My husband (Luay)

To my second family. My husband's father and my husband's mother

To my happiness of life. My sister (Hamsa)

To the source of support. My brother (Ali)

To my friend, then my sister, then the closest people to me. My husband's sister (Ghasaq)

To my kind sister. brother's wife (Zainab)

Zainab

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Zainab

Summary

Diarrhea caused by enteric infections is a major cause of morbidity and mortality. Infectious diarrhea affects an estimated 2 for 4 billion people each year, with newborns being the most vulnerable. Diarrhea is caused by bacteria, viruses and parasites.

The current study was conducted to detect the rate of infection with the parasite *B. hominis* in children under the age of ten years in patients with gastroenteritis in Diyala governorate using two detection methods, the first is the culture method and the second method is the conventional polymerase chain reaction PCR, to determine of the genetic subtypes for *B. hominis* parasite, in addition to study the relationship between infection rate and various factors such as gender, age, mother's education level, water sources, animal husbandry and clinical sign.

A cross-sectional study was conducted on children with diarrhea at Al-Batool Teaching Hospital in Baqubah city, during the period from November 2020 to April 2021, a total of 100 children under the age of ten (55 males and 45 females). Stool samples were collected and examined by culture method, the rest of the samples were preserved by deep freezing until the use of the conventional polymerase chain reaction.

The rate of infection with *B. hominis* parasite according to the culture method was 20% (20 out of 100 samples). The infection was higher among females (28.9%) compared to males (12.7%). The positive result were higher in the age group less than two years (19.8%), (23.1%) among the positive results for those who lived in Baqubah district and (9.1%) for those in neighboring districts. In this study, the highest percentage of incomplete primary mothers and primary education was reached (32%) and (17.1%) respectively and the lowest percentage of women with higher education (5.9%). As for water sources, the highest percentage of

those who used boiled tap water was (37.1%), and the lowest percentage of those who used the filtered water (4.3%). The study showed that the highest percentage was with those who kept animals at homes (36.4%), and at least for those who didn't have animals (11.9%). The children were suffering from abdominal pain (21.8%) and vomiting (21.1%) at the highest rate.

As for the results using polymerase chain reaction (PCR), the rate of infection with the parasite *B. hominis* was according to this method (8%), (8 out of 100). The infection was higher among females (11.1%) than to males (5.5%). The positive result was higher in the age group less than two years (7.4%), (7.7%) among the positive results for those who lived in Baqubah district and (9.1%) for those in neighboring districts. In this study, the highest percentage occur with patient whose mothers were incomplete primary education was reached (12%), the lowest percentage of women with higher education (5.9%). As for water sources, the highest percentage of those who use boiled tap water (14.3%), and the lowest percentage of those who use the filtered water (4.3%). The study showed the highest percentage was with those who kept animals at homes was (18.2%), and at least for those who didn't have animals as (3%). The children were suffering from vomiting (9.2%) and abdominal pain (8%) at the highest rate.

According to the genetic analysis of the sequence of eight samples that were positive for *Blastocystis hominis* parasite using the conventional polymerase chain reaction and they were back to the subtypes 3.

List of Contents

Contents		Page No.
Dedication		
Acknowledgment		
Summary		I
List of contents		III
List of tables		VI
List of figures		VII
List of abbreviations		VIII
Chapter One		
1.1	Introduction	1
1.2	Aims of study	3
Chapter Two		
2.1	Historical Perspective	4
2.2	Taxonomy of <i>Blastocystis hominis</i>	4
2.3	Subtypes and Genetic of <i>Blastocystis hominis</i>	5
2.4	Routes of Transmission	6
2.5	Morphology and Life Cycle	7
2.6	Epidemiology	10
2.7	Clinical Manifestation and Pathogenesis	11
2.8	Immunity	13
2.9	Diagnosis	15
2.10	Treatment and Prevention	16
Chapter Three		
3.1	Patients	18
3.1.1	Study Design	18
3.1.2	Sample Collection	18
3.2	Materials	19
3.2.1	Laboratory Devices and Tools	19
3.2.2	Chemical Materials	20
3.2.3	Culture Media	20
3.2.4	Kits	20
3.2.5	Materials of Gel electrophoresis	22
3.3	Methods	23
3.3.1	Culture Media Assay	23

3.3.2	DNA Extraction Procedure	23
3.3.3	Primer Preparation	26
3.3.4	Gene Amplification by Conventional PCR	26
3.3.5	Protocol of Conventional PCR	26
3.3.6	Preparation of Agarose Gel	27
3.3.7	DNA Loading and Electrophoresis Procedure	27
3.3.8	Sequencing of PCR Products and Data Analysis	27
3.4	Statistical Analysis	28
Chapter Four		
4.1	Results of <i>Blastocystis hominis</i> Infection According to the Cultured on DMEM Medium	29
4.1.1	Distribution of <i>Blastocystis hominis</i> Infection	29
4.1.2	Distribution of <i>Blastocystis hominis</i> According to Demographic Characteristic	30
4.1.3	Distribution of <i>Blastocystis hominis</i> According to Water Source	31
4.1.4	Distribution of <i>Blastocystis hominis</i> According to the Presence of Animals in House or Nearby	31
4.1.5	Distribution of <i>Blastocystis hominis</i> According to the Clinical Signs	32
4.2	Results of <i>Blastocystis hominis</i> Infection According to Conventional PCR	33
4.2.1	Rate of <i>Blastocystis hominis</i> Infection	33
4.2.2	Rate of <i>Blastocystis hominis</i> According to Demographic Factors	34
4.2.3	Rate of <i>Blastocystis hominis</i> According to Source of Water	35
4.2.4	Rate of <i>Blastocystis hominis</i> According to the Presence of Animals in House or Nearby.	35
4.2.5	Rate of <i>Blastocystis hominis</i> According to the Clinical Signs	36
4.3	Genetic Analysis of <i>Blastocystis hominis</i>	36
4.3.1	Alignment of <i>Blastocystis hominis</i> Sequences with Reference Sequence	36
4.3.2	Submission of Local Iraq Isolate in NCBI	37
4.3.3	Phylogenetic Tree	38

4.3.4	Multiple Sequences	39
	Chapter Five	
5.1	Rate of <i>Blastocystis hominis</i> Infection According to the Cultured on DMEM Medium	42
5.2	Rate of <i>Blastocystis hominis</i> Infection According to PCR	42
5.3	Rate of <i>Blastocystis hominis</i> Infection According to Gender	44
5.4	Rate of <i>Blastocystis hominis</i> Infection According to Age	44
5.5	Rate of <i>Blastocystis hominis</i> Infection According to Residence	45
5.6	Rate of <i>Blastocystis hominis</i> Infection According to Mother's Educational Level	45
5.7	Distribution of <i>Blastocystis hominis</i> Infection According to Source of Water	46
5.8	Distribution of <i>Blastocystis hominis</i> Infection According to Presence of Animals in House or Nearby	47
5.9	Rate of <i>Blastocystis hominis</i> Infection According to Clinical Sign	48
5.10	Genetic Analysis	49
	Chapter Six	
6.1	Conclusions	51
6.2	Recommendations.	52
	References	53-68
	Appendix	

List of Tables

Table	Title	Page No.
3-1	Devices and tools used in this study	19
3-2	Chemical materials that used in this study	20
3-3	Quick-DNA™ fecal/soil microbe miniprep kit components	21
3-4	Components of the maxime PCR PreMix kit (i-Taq)	21
3-5	Sequence of primers used in this study	26
3-6	The condition of thermal cycling for DNA amplification	26
4-1	Distribution of <i>Blastocystis hominis</i> according to demographic characteristic	30
4-2	Distribution of <i>Blastocystis hominis</i> according to water source	31
4-3	Distribution of <i>Blastocystis hominis</i> according to the presence of animals in house or nearby	31
4-4	Distribution of <i>Blastocystis hominis</i> according to the clinical signs	32
4-5	Rate of <i>Blastocystis hominis</i> according to demographic factors	34
4-6	Rate of <i>Blastocystis hominis</i> according to source of water	35
4-7	Rate of <i>Blastocystis hominis</i> according to the presence of animals in house or nearby	35
4-8	Rate of <i>Blastocystis hominis</i> according to the clinical signs	36
4-9	Represent type of polymorphism of <i>Blastocystis hominis</i> Subtype 3 small subunit ribosomal RNA gene in the local isolates and related with the reference isolate from GenBank (indicated by their accession numbers)	37
4-10	Compatibility of Iraqi local isolates with 13 global isolates from Gene Bank	39

List of Figures

Figure	Title	Page No.
2-1	Morphological forms of <i>Blastocystis</i> : (a, b) vacuolar form, (c) granular form unstained, (d) granular form stained with methylene blue, (e-g) cyst-like form unstained, in iodine stain, and lactophenol cotton blue stain, , (h and i) Ameboid form	8
2-2	Life cycle of <i>Blastocystis</i>	10
3-1	Quick protocol diagram for DNA extraction	25
4-1	Rate of <i>Blastocystis hominis</i> infection according to the culture	29
4-2	Three morphological forms of <i>Blastocystis hominis</i> in DMEM medium stained with iodine stain: (A, B) vacuolar form, (C) granular form, (D) cyst form	29
4-3	Rate of <i>Blastocystis hominis</i> infection according to conventional PCR	33
4-4	Image of gel electrophoresis shows the positive bands for the SSU rRNA gene of <i>Blastocystis hominis</i> , stained with red safe stain on 2% agarose gel, M: ladder marker (100-1000bp)	33
4-5	Neighbor-joining tree <i>Blastocystis hominis</i> Subtype 3 small subunit ribosomal RNA gene, involved 21 nucleotide sequences (13 sequences reference from Gene Bank). Current isolates are indicated with red triangle	38
4-6	Multiple sequences of <i>Blastocystis hominis</i> subtype 3 small subunit ribosomal RNA gene	41

List of abbreviations

Abbreviation	Meaning
DMEM	Dulbecco's modified eagle medium
DNA	Deoxyribonucleic acid
EtBr	Ethidium bromide
GM-CSF	Granulocyte-macrophage colony- stimulating factor
HIV	Human immunocompromised virus
IBS	Irritable bowel syndrome
IDT	Integrated DNA technologies
IFN- γ	Interferon gamma
IL	Interlukin
NCBI	National Center Biotechnology Information
NHPs	Non-human primates
°C	Centigrade
ORS	Oral rehydration solution
PCR	Polymerase chain reaction
SAS	Statistical Analysis System
SSUr DNA	Small subunit ribosomal DNA genes
SSUr RNA	Small subunit ribosomal RNA genes
STs	subtypes
TBE	Tris-Borate-EDTA
Th	T-helper cell
TNF- α	Tumor necrosis factor alpha
UAE	United Arabian Emirates

Chapter One

Introduction

1.1 Introduction

Parasitic infection, especially intestinal parasites, is considered as the most common communicable diseases worldwide, especially in developing countries (Belete *et al.*, 2021). Globally about 3.5 billion people are affected, 450 million complain as a result of these infections, and the mostly age groups affected are children (Barati *et al.*, 2021).

The most prominent pathogenic human intestinal protozoans are: *Entamoeba histolytica*, *Blastocystis sp.*, *Giardia intestinalis* and *Dientamoeba fragilis*. *Blastocystis sp.* is a highly prevalent suspected pathogenic protozoan, and considered an unusual protist due to its significant genetic diversity and host plasticity (Arbat *et al.*, 2018; Barbosa *et al.*, 2018).

Blastocystis is a common anaerobic protist living in many animals' and humans' gastrointestinal tracts and it is taxonomically placed within the Stramenopiles (Ramírez *et al.*, 2014).

Blastocystis species, the non-motile Stramenopile, isolated from human gut, has four various morphological forms i.e. granular, vacuolar, ameboid and cystic. Among these, vacuolar is the most commonly isolated from human stool specimens. In less than a decade, 17 different subtypes (STs) of *Blastocystis sp.* have been described on the basis of Sequence Tagged Sites (STS) analysis on small subunit ribosomal RNA genes (SSU-rRNA) locus (Alfellani *et al.*, 2013; Das *et al.*, 2016).

Blastocystis hominis is associated with different gastrointestinal disorders as diarrhea, abdominal pain, fatigue, constipation, flatulence and irritable bowel syndrome (IBS) (Kurt *et al.*, 2016), while found in asymptomatic persons as a commensal parasite (Lepczyńska *et al.*, 2017).

Blastocystis hominis has a worldwide distribution and is considered one of the most common human protozoa especially in developing countries with a higher prevalence rate (30%–50%) than in developed countries (1.5% –10%) (Eassa and Masry, 2016).

Several studies were conducted to determine the rate of *Blastocystis hominis* infections in Iraq, using different techniques such as study done by Ridha and Faieq, (2021) in Wasit province who recorded that (67.12%), each sample was examined using both normal saline and Lugol's iodine preparation by direct wet mount microscopic examination, Merza *et al.*, (2020), in Duhok city, Kurdistan region used the culture method where the percentage (16.93%). Hasan and Al-Samarrai, (2020) in Baghdad showed that the rate of *Blastocystis hominis* was (22.5%) they used examination under direct microscopy, while a study conducted in Diyala province used serological diagnosis showed the percentage (32%) (Alazzawi *et al.*, 2020). In this study, the percentage of *Blastocystis hominis* infection was determined by using two methods, culture and polymerase chain reaction (PCR) within the age group of less than 10 years.

1.2 Aims of Study

1. To detect the infection rate of *Blastocystis hominis* in children under 10 years old with diarrhea in Diyala.
2. To study and compare two methods of diagnosis, culture method and polymerase chain reaction (PCR) method for the detection of *Blastocystis hominis* and sequencing the product of the positive result
3. To determine the association between *Blastocystis hominis* infection and different factors such as gender, age, residence, the level of mother education, water source, the presence or absence animals in their houses and clinical sign.