

Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Diyala
College of Medicine



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

A Thesis

Submitted to Council of College of Medicine - University of Diyala
as Partial Fulfillment of the Requirements for the Master's Degree
of Sciences in Medical Microbiology

By
Zainab Naseef Jassim

BSc. (2016) - College of Science - University of Diyala

Supervised by

Assistant Professor
Dr. Mohammed J. Shaker

Professor
Dr. Mehdi Sh. Al-Zuheriy

2021 A.D.

1443 A.H.

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

ACKNOWLEDGEMENTS

To “ALLAH” and to his prophet “Mohammed” so my thanks and gratitude for giving me strength. Without them, this research would not have been achieved.

I would like to express my thanks and appreciation to assistant Professor Dr. Mohammad Jassim shaker and Professor Dr. Mehdi SH. AL-Zueriry for their help and guide me throughout the course of preparing my thesis.

My thanks go to dean of College of Medicine and to the staff of Microbiology Department for providing the chance to get the degree of Master in Microbiology.

I would also like to thank the staff of the Department of Medical Laboratories at AL-Batool Hospital for their provide ongoing assistance, especially, biologist Rana Ahmed Abdel-Lateef, biologist Solafa Reda Salman, and bacteriology Ahmed Salman Yas.

Special thank goes to assistant teacher Massar Hadi Ismail To help me provide useful information.

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.

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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.