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Molecular Detection of Human Bocavirus among Children with Gastroenteritis in Diyala Governorate

A Thesis

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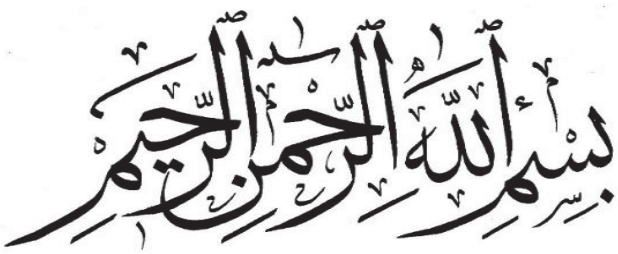

﴿وَتِلْكَ الْأَمْثَالُ نَضْرِبُهَا لِلنَّاسِ وَمَا يَعْقِلُهَا إِلَّا الْعَالِمُونَ﴾

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We, certify that this thesis entitled **(Molecular Detection of Human Bocavirus among Children with Gastroenteritis in Diyala Governorate)** has been conducted under our supervision at College of Medicine - University of Diyala, as a partial fulfillment of the requirements for the Master Degree of Science in Medical Microbiology.

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Dedication

I dedicate this work to

- *To the source of inspiration.* ***My father****.*
- *To the reason for my happiness in life.* ***My mother****.*
- *To my strength and my ideal in life.* ***My brothers (Ghassan and Luay).***
- *To the good-hearted, my new sister.* ***Zainab*** *(my brother's wife) Thank God for being near me.*
- *To my friends and everyone who loves me with all his heart and wishes good for me.*

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Summary

Acute gastroenteritis is the major public health problem among infants and children worldwide and highly mortality and mortality were recorded in developing countries. Human bocavirus consist of four species, first one associated with respiratory tract infection while others have been described as enteric viruses mainly excreted in stool.

The current study is designed to determine the rate of human bocavirus infection among children less than 5 years of age with gastroenteritis in Diyala governorate using conventional polymerase chain reaction, to identify the human bocavirus genotypes, in addition to study the correlation between the rate of infection and different parameters such as age, gender, the education level of the mothers, water source, type of feeding and clinical aspects.

A cross sectional study was carried out for patients with acute gastroenteritis who attended the Emergency Department of Pediatrics in Al-Batool Teaching Hospital for Maternity and Pediatric in Baqubah city, during the period from July 2019 to March 2020. A total of 100 children under the age of five years old (58 males and 42 females). The stool samples were collected from each patient and stored at -70 °C until using conventional PCR to identify of HBoV genotypes.

The rate of HBoV infection according to the results of this study was 9%, the infection was higher (10.34%) in males rather than females (7.14%). The positive result in age group (6-12 months) was 5 (12.5%). Seven cases of the positive results were from Baqubah districts while 2 positive cases from other districts like Al-Khalis and Balad Ruz. The maternal's educational level for patients were (9.09%) for illiterates and

(12.12%) primary education, followed by (8.33%) with secondary education, while no positive result with high education.

The distribution of positive human bocavirus infection according to type of feeding showed that 4 cases (12.9%) were used mixed feeding, 3 cases (5.36%) were used artificial milk and 2 cases (18.18%) were used breast feeding while no infection noticed with children used food only. The highest infection rate was 4(13.33%) for groups of children use filtered water and 4(8%) use boiled filtered water.

The positive children for human bocavirus presented with different gastrointestinal signs high frequency noticed with weight loss 9(100%) abdominal pain 7(77.77%) and vomiting 6(66.66%).

According to the genetic analysis of the sequence of four samples (4 out of 9 positive cases for conventional polymerase chain reaction), three of them belong to human bocavirus type 3, while the fourth related to human bocavirus type 2.

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List of abbreviations

Abbreviation	Meaning
AGE	Acute gastroenteritis
BPV1	Bovine parvovirus 1
ºC	Centigrade
CD4	Cluster of differentiation 4
CDC	Centers for disease control
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
EtBr	Ethidium bromide
FBoV	Feline bocavirus
HBoV	Human bocavirus
ICTV	International Committee on Taxonomy of Viruses
IFN-β	Interferon Beta
IFN-γ	Interferon gamma
IL	Interlukin
IRF3	Interferon regulatory factor-3
mRNA	Messenger RNA
MVC	Minute virus of canines
NK cells	Natural killer cells
NLS	Nuclear localization signal
NP1	Nuclear phosphoprotein
NS1	Nonstructural protein
OAS	Original antigenic sin
ORFs	Open reading frames
ORS	Oral rehydration solution
PBoV	porcine bocavirus
PCR	Polymerase chain reaction
PLA2	Phospholipase A2
PPV4	porcine parvovirus 4
ssDNA	Single stranded DNA
Th	T-helper cell
TNF-α	Tumor necrosis factor alpha
VLPs	Virus-like particles
VP1-2	Capsid viral proteins 1-2
VP1u	Unique N-terminal region of VP1
WHO	World health organization

Chapter One
Introduction

1.1 Introduction

Acute gastroenteritis (AGE) is still a major cause of morbidity and mortality in infants and children around the world. A child younger than 5 years may experience as many as 1 to 5 episodes of acute diarrhea each year. Diarrheal diseases remain the second cause of death among children <5 years old, mostly in low- and middle-income countries. According to the centers for disease control (CDC), viral gastroenteritis infections can account for over 446,000 deaths of children per year worldwide (Guarino *et al*., 2020).

Acute gastroenteritis is a clinical syndrome often defined by increased stool frequency (Onset 3 or more loose or watery stools in 24 hours or a number of loose/watery bowel movements that exceeds the child's usual number of daily bowel movements by two or more), with or without vomiting, fever, or abdominal pain (Bányai *et al*., 2018).

Acute childhood diarrhea or acute gastroenteritis is often associated with human enteric viruses belonging to different taxonomic groups. Many viruses such as rotavirus, adenovirus, human norovirus, human astrovirus, and sapovirus have been known to associate with these diseases and also, human Bocavirus (HBoV) has been considered as agent associated with diarrhea in humans (Platts-Mills *et al*., 2015; Zhirakovskaia *et al*., 2019).

Human bocaviruses belong to the family *Parvoviridae,* subfamily *Parvovirinae*. The viral particles are small non-envelop, icosahedral capsid. Genome of HBoV is a direct single deoxyribonucleic acid (DNA) that encodes two nonstructural proteins and two capsid viral proteins 1, 2 as structural proteins (Peltola *et al*., 2013).

Human bocavirus is the second human pathogen parvovirus and divided into four species; HBoV-1 has been predominantly diagnosed in the respiratory tract whereas the three other types, HBoV-2, HBoV-3 and HBoV-4, have been described as enteric viruses mainly excreted in stool (Soares *et al*., 2019).

Patients infected by the human bocavirus develop various clinical signs, such as rhinitis, pharyngitis, cough, dyspnea, wheezing, pneumonia, acute otitis media, fever, nausea, vomiting and diarrhea. However, HBoV can also be detected in an asymptomatic people (Jartti *et al*., 2012). Human bocavirus VP2 virus like particle (VLPs) have good immunogenicity with induction of strong humoral and cellular immune responses (Deng *et al*., 2014).

The main methods of diagnosing human bocavirus by conventional PCR and real time PCR, may be due to the little success of the serological and cultural techniques of the virus (Rikhotso *et al*., 2018).

Two studies have been conducted in Baghdad such as Atyah *et al*., (2017) and Hasan *et al*., (2018), focusing on the relationship between human bocavirus and respiratory tract infections, while several studies were done in different places outside Iraq to detect rate of human bocavirus infection in stool such as study done by El-Mosallamy *et al*., (2015) in Egypt who recorded that 2%, Lekana-Douki *et al*., (2018) in Gabon was 2.2%, Monavari *et al., (*2013) in Iran who found that out of 200 patient the infection rate was 8% positive for HBoV other study done by Nora-Krukle *et al*., (2018) found 21.2% in Switzerland. Up to our knowledge there is no Iraqi study done in Baqubah city and focused in relationship between human bocavirus and gastroenteritis, so the present study designed for this purpose.

1.2 Aims of study

1. To determine the infection rate of human bocavirus in children under five years old with gastroenteritis in Diyala governorate.
2. To study the molecular identification of human bocavirus genotypes in study population.
3. To study the correlation between human bocavirus infection and different parameters such as age, gender, residence, the level of maternal education, water source, types of feeding and clinical aspects.