DETECTION AND CHARACTERIZATION OF HEPATITIS A VIRUS CIRCULATING IN EGYPTIAN ENVIRONMENT

By

HAZEM AHMED HAMZA EWESS

B.Sc. Agric. Sci. (Agricultural Biochemistry), Fac. Agric., Cairo Univ., 2006

THESIS

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APPROVAL SHEET

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SUPERVISION SHEET

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Name of Candidate: Hazem Ahmed Hamza Ewess **Degree:** M.Sc.
Title of Thesis: Detection and characterization of Hepatitis A virus circulating in Egyptian environment

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ABSTRACT

Hepatitis A virus (HAV) is an important causative agent of acute hepatitis in humans and still poses a considerable problem worldwide. It is primarily transmitted through fecal- oral route by consumption of contaminated food or water. Over one year of survey, HAV was recovered from wastewater samples that were collected from three wastewater treatment plants in greater Cairo (Zeinin, El-Berka and Balaks). HAV was detected in 27 out of 68 samples (39.7%) using RT-PCR represent both influent and effluent. Eleven positive samples were subjected for sequencing targeting VP1-2A junction region. Phylogenetic analysis revealed that all samples belong to subgenotype IB. The complete nucleotide sequence of one isolate (HAV/Egy/BI-11/2015) showed that the similarity over the amino acid level wasn't reflected at the nucleotide level. However, the deuced amino acids of the complete nucleotide sequence showed a distinct substitution at 2B, 2C, and 3A regions. The recombination analysis revealed a recombination event (nucleotide 3757- 3868) involving a portion of 2B nonstructural protein coding region using specific software assuming X75215(GBM), subgenotype IA, as an actual recombinant. Despite the role of recombination in Picornavirues evolution, its involvement role in HAV evolution was reported in few reports and may be due to the limited number of complete HAV sequences. These data constitute an update for the pattern of HAV circulating in Egypt and a first available complete nucleotide sequence data for an Egyptian isolate.

Keywords: Hepatits A virus, full-length genome, wastewater, RT-PCR, Genotyping.

DEDICATION

To My

Parents

Brothers & Sisters

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CONTENTS

LIST OF ABBREVIATIONS

AGM	African Green Monkey
AGMK	African Green Monkey Cells
AH	Acute Hepatitis
CDC	Center for Disease and Control
CPE	Cytopathic Effect
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethylsulfoxide
dNTP	Deoxynucleoside triphosphates
EDTA	Ethylenediaminetetraacetate
FBS	Fetal Bovine Serum
FH	Fulminant Hepatitis
FRhK-4	Fetal Rhesus kidney Cells
HAV	Hepatitis A Virus
Huh-7	Human Hepatoma Cells
ICC-PCR	Integrated Cell Culture Polymerase Chain Reaction
IRES	Internal Ribosomal Entry Site
Kb	Kilobase
M-MLV	Moloney Murine Leukemia Virus Reverse Transcriptase
NASBA	Nucleic acid Sequence Based Amplification
nt	Nucleotide
ORF	Open Reading Frame
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
qPCR	quantitative Real Time Polymerase Chain Reaction
RDP	Recombination Detection Program
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
TAE	Tris-acetate- EDTA Buffer
UTR	Untranslated Region
VIRADEL	Virus Adsorption-Elution
WHO	World Health Organization
wt-HAV	wild type Hepatitis A Virus
WWTP	Wastewater Treatment Plant