

Ain Shams University
Faculty of Science
Microbiology Department



**Isolation and Genotyping characterization
of Group A rotavirus among Children with
Severe diarrhea**

By

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(M.Sc. in Microbiology-2005)

Thesis

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List of Abbreviations

A:	Adenine
A ⁰ :	Angstrom
AMP:	Adenosine monophosphate
Bp :	Base pairs
C:	Cytosine
cDNA:	Complimentary DNA
CPE:	Cytopathogenic effect
C ⁰ :	Centigrade
DEPC:	Diethyl pyrocarbonate
EMEM:	Eagle Minimum essential medium
DNA:	Deoxyribonucleic acid
dNTPs:	Deoxyribonucleotide triphosphates
dsRNA:	Double strand RNA
EDTA:	Ethylenediamine tetra acetic acid
EGTA:	Ethylene glycol tetraacetic acid
EIA:	Enzyme Immuno Assay
ELISA:	Enzyme Linked Immunosorbent Assay
ER:	Endoplasmic Reticulum
EM:	Electron Microscope
EPI:	Expanded Program for Immunization
FBS:	Foetal Bovine Serum
G:	Guanine
GAVI:	Global Alliance for Vaccines and Immunization
gm:	gram
HIV:	Human immunodeficiency virus
ICC-RT-PCR:	Integrated cell culture RT-PCR
MA104:	Monkey African green kidney

mRNA:	Messenger RNA
ml:	milliliter
Mm:	Millimole
MAB:	Monoclonal antibodies
μl:	Micro liter
nm:	Nano-meter
NSP:	Non-structural protein
nt:	Nucleotide
ORF:	Open Reading Frame
PBS:	Phosphate buffer saline
PAGE:	Polyacrylamide gel electrophoresis
pmol:	picomole
PCR:	Polymerase Chain Reaction
RNA:	Ribonucleic acid
RT-PCR:	Reverse transcription- Polymerase chain reaction
RV:	Rotavirus
SA11:	Simian rotavirus
T:	Thamine
TAE:	Tris-Acetate-EDTA
U:	Unit
US:	United State
VP4:	Protein (protease sensitive protein)
VP7:	Protein (glucose sensitive protein)
W/V:	Weight/Volume
WHO:	World Health Organization

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Chapter I

Introduction

Diarrheal disease kills 1.8 million children <5 years of age annually (*WHO, 2009*). It is the fourth most common cause of death and accounts for 16% of all deaths in this age group (*Bryce et al., 2005, Parashar et al., 2006*). Rotavirus is the most common agent responsible for severe diarrheal disease (*Glass and Parashar, 2006*), causing 0.6 million deaths, 2.4 million hospitalizations, 24 million outpatient visits, and 114 million episodes of diarrhea per annum (*Glass et al., 2006*). Rotavirus was first identified as an important etiological agent in childhood diarrhea in 1973 (*Bishop et al., 1973*). Two new safe and effective vaccines have been widely licensed internationally. This holds great promise to decrease the mortality and morbidity associated with diarrheal disease in developing countries (*Glass and Parashar, 2006*). The 2 new internationally licensed vaccines, Rotarix (Glaxo- SmithKline Biologicals) and

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RotaTeq (Merck), underwent 2 of the largest prelicensure efficacy and safety trials (*Ruiz-Palacios et al., 2006, Vesikari et al., 2006*). Rotarix was first licensed in December 2004 in Mexico and in February 2006 in the European Union, and RotaTeq was approved by the US Food and Drug Administration in February 2006. Rotarix attained prequalification status from the World Health Organization (WHO) in February 2007, and RotaTeq did so in October 2008. The GAVI Alliance has approved vaccine purchase since 2008 in Latin America and Europe, where the vaccines have been shown to be efficacious in randomized controlled clinical trials (*Ruiz-Palacios et al., 2006*). The WHO Strategic Advisory Group of Experts met in April 2009 and reviewed the data from efficacy studies conducted in Africa and has recommended the universal inclusion of rotavirus vaccines in national immunization programs (*WHO, 2009*).

Rotavirus infection is of special significance in developing countries where they constitute a major cause of mortality among the young children. Although

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diarrheal diseases are not a prominent cause of mortality in infants and young children in developed countries, the toll from diarrheal disease in developing countries is staggering. Due to the lack of proper disease management and/or the unavailability of health care facilities in the developing world, rotavirus kills over half a million infants a year, about one child each minute (*Newsrx, 2006*).

Rotavirus infection causes ~527,000 deaths per year worldwide; however, the large proportion of this mortality occurs in the lowest-income countries, and almost half (~230,000) of all deaths worldwide are estimated to occur in Africa (*Parashar et al., 2006, WHO, 2009*). However, only very limited direct data on rotavirus disease burden are available from sub-Saharan Africa to validate these estimates (*Molbak et al., 2000*).

Furthermore, diarrheal illnesses consistently rank as one of the top six causes of all deaths, one of the top three causes of death from an infectious disease (*Murray and Lopez, 1997*).