Genetic Variability of *Cryptosporidium* Isolates from Humans in Greater Cairo, Egypt

Thesis

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To my lovely family and friends

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LIST OF ABBREVIATIONS

AF	Acid-fast
AFLP	Amplified fragment length polymorphism
AIDS	Aquired immunodeficiency syndrome
AP	Auramine-phenol
AP-PCR	Arbitrary primed-PCR
Bp	base pair
°C	Degree Celsius
C.	*Cryptosporidium*
CDC	Center of disease control
CDPKs	Calcium-dependent protein kinases
COWP	*Cryptosporidium* oocyst wall protein
DAPI	4',6-diamidino-2-phenylindole
ddATP	dideoxyadenosine triphosphate
ddCTP	dideoxycytidine triphosphate
ddGTP	dideoxyguanosine triphosphate
ddNTP	dideoxynucleotide-tri-phosphate
ddTTP	dideoxythymidine triphosphate
DFA	Direct fluorescent-antibody
DNA	Deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
dsDNA	Double strand Deoxyribonucleic acid
EIAs	Enzyme immunoassays
ELISA	Enzyme linked immunosorbent assay
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
FITC-C-mAB	Flourescein isothiocyanate-conjugated anti-*Cryptosporidium* monoclonal antibody
G	Gram
G. duodenalis	*Giardia duodenalis*
Gp60	Glycoprotein 60
H & E	Hematoxylin and Eosin
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HSP	Heat shock protein
ICT	Immuochromatographic test
IFA	Immunoflourescent assay
IFN-γ	Interferon-gamma

IgA	Immunoglobulin A
IgM	Immunoglobulin M
IL	Interleukin
IQR	interquartile range
K	Potassium
KDa	Kilodalton
LAMP	Loop Mediated Isothermal Amplification
mAbs	Monoclonal antibodies
ml	Milliliter
mZN	modified Ziehl-Neelsen
NK	Natural killer cells
nPCR	Nested polymerase chain reaction
№	Number
PCR	Polymerase chain reaction
PVA	Polyvinyl alcohol
qPCR	Quantitative real-time PCR
RAPD-PCR	Random amplified polymorphic DNA-PCR
RFLP	Restriction fragment length polymorphism
rRNA	ribosomal Ribonucleic acid
RT	Real-time
RT-PCR	reverse transcription-PCR
s	Second
SAF	Sodium acetate formaldehyde
SD	Standard deviation
Sig.	Significance
Spp.	Species
ssDNA	Single stranded DNA
SSU rRNA	Small-subunit ribosomal Ribonucleic acid
TNF-α	Tumor necrosis factor-alfa
TRAP	Thrombospondin-related adhesive proteins
µl	microliter
µm	Micrometer
U	Units
USA	United States of America
UV	Ultra-violet
WHO	World health organization

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ABSTRACT

Background: Human cryptosporidiosis caused by *Cryptosporidium* parasite has been recognized worldwide as the most common cause of protozoal diarrhea leading to significant morbidity and mortality in industrialized nations and developing countries. The vast majority of human cases of cryptosporidiosis worldwide are caused by two species: *Cryptosporidium hominis*, which causes infection in humans only, and *Cryptosporidium parvum* which causes infections in humans and animals. Exposition of *Cryptosporidium* genotypes by molecular assays is required to recognize sources of infections and routes of transmission, facilitating the improvement of risk assessment and measures for prevention and control**.** The aim of the present study was to detect and correlate native *Cryptosporidium* molecular genetic variability among human isolates in Greater Cairo, Egypt, with their respective demographic, environmental and clinical manifestations.

Method: A total of 350 human stool samples collected from Egyptian patients from Greater Cairo, Egypt, with variable demographic, environmental and clinical presentations were subjected to modified Ziehl-Neelsen stain. Then, they were examined by RIDA QUICK *Cryptosporidium/Giardia* immunochromatography kit. All positive *Cryptosporidium* samples diagnosed by stain and/or kit were processed for Deoxyribonucleic acid (DNA) extraction. The extracted DNA samples were genotyped using nested polymerase chain reaction-Restriction fragment length polymorphism (nPCR-RFLP) targeting Small-subunit ribosomal Ribonucleic acid (SSU