COMPARATIVE EVALUATION OF REAL TIME MULTIPLEX PCR, COPRO-ANTIGEN DETECTION ASSAYS AND MICROSCOPY FOR THE DIAGNOSIS OF *ENTAMOEBA HISTOLYTICA, GIARDIA INTESTINALIS* AND *CRYPTOSPORIDIUM* SPECIES INFECTIONS

THESIS SUBMITTED TO

Faculty of Medicine, Ain Shams University
for partial fulfillment of MD in medical Parasitology

BY

John Talaat Nazeer
M.B, B.Ch. MSc
Assistant lecturer of Parasitology
Faculty of Medicine, Ain Shams University

SUPERVISORS

Prof. Dr. Mahmoud Mohamed El-Sibaei
Professor of Parasitology
Faculty of Medicine, Ain Shams University

Prof. Dr. Magda Youssef Abdel-Hamid
Professor of Parasitology
Faculty of Medicine, Ain Shams University

Prof. Dr. Khalifa El-Sayed Khalifa
Professor of Parasitology
Faculty of Medicine, Ain Shams University

Prof. Dr. Egbert Tannich
Professor and Chairman, Head of Molecular Parasitology Department
Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Dr. Ranya Ayman Samir Tawfik
Assistant professor of Parasitology
Faculty of Medicine, Ain Shams University

Parasitology Department
Faculty of Medicine, Ain shams University
2014

مقارنة تقييمية لتفاعل البلمرة التسلسلي الوقتى المتعدد، اختبار الانتيجين فى البراز والفحص الميكروسكوبى لتشخيص عدوى الانتاميبا هستوليتيكا، الجياردیا المعوية والكريبتوسبوريديوم

موضوع رسالة مقدم الى كلية الطب جامعة عين شمس
توطئة للحصول علي درجة الدكتوراه في العلوم الطبية الاساسية
(علم الطفيليات)

مقدم من

الطبيب/ چون طلعت نظير

مدرس مساعد بقسم علم الطفيليات
كلية الطب – جامعة عين شمس

المشرفون

الأستاذ الدكتور/ محمود محمد السباعي
الأستاذ بقسم علم الطفيليات
كلية طب – جامعة عين شمس

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كلية طب – جامعة عين شمس

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أستاذ ورئيس قسم البيولوجيا الجزيئية لعلم الطفيليات بمعهد برنهارد نوخت لطب المناطق الحارة، هامبورج، المانيا

الدكتورة/ رانية أيمن سمير توفيق
الأستاذ المساعد بقسم علم الطفيليات
كلية الطب – جامعة عين شمس

قسم علم الطفيليات
كلية الطب – جامعة عين شمس

2014

Acknowledgement

First of all, thanks to God, to whom I relate any success that can be achieved at work in my life.

Words are not enough to express how deeply grateful I am to ***Prof Dr. Mahmoud Mohamed El-Sibaei,****Professor of Parasitology, Faculty of Medicine, Ain Shams University, for his immense support and much of his time for this work to be done.*

I am greatly honored to express my high appreciation and gratitude to ***Prof. Dr.MagdayoussefAbdel-Hamid,*** *Professor of Parasitology, Faculty of Medicine, Ain Shams University, for her gracious supervision, precious advice, and continuous encouragement through the whole research.*

*Words can never express my gratitude and thanks to****Prof.Dr. KhalifaElsayedKhalifa****, Professor of Parasitology, Faculty of Medicine, Ain Shams University, for his kind help, meticulous supervision and continuous support and guidance throughout this work.*

Also I would like to express my gratitude and appreciation to ***Prof. Dr.Egbert Tannich****, Head of Molecular Parasitology Department Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, for his fruitful encouragement, vast knowledge and valuable support.*

I am heartily thankful to ***Dr. RanyaAyman Samir****, Assistant Professor of Parasitology for her guidance and support from the initial to the final level.*

*Actually I'd like to show my gracefulness for the kind financial support by the Institute for Quality Assurance in Laboratory Medicine "****INSTAND e.v****.", Dusseldorf, Germany.*

Finally, I wish to thank all my family especially my wife, all my colleagues and any member who shared in this modest piece of work for their cooperation and patience.

Abstract

Diarrhea is an important cause of morbidity and mortality, worldwide. *Giardia intestinalis*, *Cryptosporidium* spp. and *Entamoeba histolytica* are the most common diarrhea-causing parasitic protozoa. Diagnosis of these parasitic infections is usually performed by microscopy. However, microscopy lacks sensitivity and specificity. One important alternative is the development of an antigen capture ELISA for use with stools. These tests that are fairly simple to perform and do not require the observation of intact organisms, have shown comparable sensitivity to experienced microscopic examinations. Replacing microscopy with more sensitive and specific nucleic acid based methods is hampered by the higher costs, in particular in developing countries. Multiplexing the detection of more than one parasite in a single test by real time PCR has been found to be very effective and would decrease the cost of the test. In the present study, stool samples collected from 396 Egyptian patients and 202 healthy controls were tested by multiplex real-time for simultaneous detection of *Entamoeba histolytica, Giardia intestinalis,* and *Cryptosporidium* spp. *Giardia intestinalis* was found to be the most common protozoan parasite causing diarrhea (37.1%) followed by *Cryptosporidium* spp. (3.0%). While, none of the diarrheal cases gave positive results for *Entamoeba histolytica*, 2.0% revealed DNA from non-pathogenic *Entamoeba dispar*. The current study has revealed that multiplex real-time PCR showed better performance when compared with microscopy and copro-antigen ELISA. Although the reagents costs are higher compared to microscopy, pooling of samples to a reference laboratory would reduce the running cost of the test which is more sensitive and specific.

Key words: Diarrhea, Egypt, Protozoa, ELISA, Multiplex, Real time PCR

List of Abbreviations

5-HT	5-hydroxytryptamine
Ag	Antigen
AIDS	Acquired Immunodeficiency Syndrome
ALA	Amoebic liver abcess
Bcl-2	B-cell lymphoma-2
CD	Cluster of differentiation
CIEP	Counter immunoelectrophoresis
Ct	Cycle threshold
DAPs	Disc-associated proteins
DFA	Direct Fluorescent Antibody
DNA	Deoxyribonucleic acid
E. disp	*Entamoeba dispar*
E. histo	*Entamoeba histolytica*
*Eh*CP	*Entamoeba histolytica* Cysteine Protinase
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
ER	Endoplasmic reticulum
Fe-Hx	Iron haematoxylin stain
FRET	Fluorescence resonance energy transfer
G	Group
GIT	Gastrointestinal Tract
H. nana	*Hymenolepis nana*
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICT	Immunochromatographic Test
IL-10	Interleukin-10
IFA	Indirect Fluorescent Antibody
IFN-γ	Interferon-γ
Ig	Immunoglobulin
IL	Interleukin
mAb	Monoclonal Antibody
Mic	Microscopy

MIF	Merthiolate (thimerosal)-iodine-formalin
min	Minutes
mRNA	Messenger Ribonucleic acid
MZN	Modified Ziehl Neelsen
No	Number
NF-κB	Nuclear Factor-κB
O&P	Ova and Parasite
PCR	Polymerase Chain Reaction
PGE2	Prostaglandin E-2
PMNs	Polymorphonuclear neutrophils
PV	Parasitophorus Vacuole
PVA	Polyvinyl alcohol
qPCR	Quantitative Real time PCR
rRNA	Ribosomal Ribonucleic acid
SAF	Sodium acetate-Acetic acid-Formalin
SEM	Scanning electron microscope
SM	Safranin methylene blue
SPCDIS	Solar photocatalytic disinfection
Spp.	Species
TEM	Transmission electron microscope
TGF-β	Transforming Growth Factor-β
TNF-α	Tumour Necrosis Factor-α
TPI	Triose phosphate isomerase
μm	Micron
UV	Ultra violet
VSP	Variant surface proteins
WHO	World Health Organisation
WT	Wheatley trichrome stain
ZO1	Zonula occludens1
ZN	Ziehl Neelsen

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