

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

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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
<i>E. disp</i>	<i>Entamoeba dispar</i>
<i>E. histo</i>	<i>Entamoeba histolytica</i>
<i>EhCP</i>	<i>Entamoeba histolytica</i> 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
<i>H. nana</i>	<i>Hymenolepis nana</i>
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- κ B	Nuclear Factor- κ B
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
<i>Spp.</i>	Species
TEM	Transmission electron microscope
TGF- β	Transforming Growth Factor- β
TNF- α	Tumour Necrosis Factor- α
TPI	Triose phosphate isomerase
μ m	Micron
UV	Ultra violet
VSP	Variant surface proteins
WHO	World Health Organisation
WT	Wheatley trichrome stain
ZO1	Zonula occludens1
ZN	Ziehl Neelsen

List of Tables

Table		Page
Table (1)	Common pathogens of acute infectious diarrhea	13
Table (2)	Oligonucleotide primers and probes used for <i>Entamoeba histolytica/ dispar</i> , <i>Giardia intestinalis</i> , and <i>Cryptosporidium</i> spp. Multiplex- real-time PCR	176
Table (3)	The Primer, probe volumes and final concentrations/ reaction	178
Table (4)	Comparison of the results of a diagnostic test with a reference golden standard using a 2x2 table	185
Table (5)	Age of cases and control group	187
Table (6)	Gender distribution among cases and control group	187
Table (7)	Clinical manifestations among cases	188
Table (8)	Stool consistency among cases	189
Table (9)	Comparison of direct smear method with formol-ethyl acetate concentration technique for the detection of enteric parasites among 396 patients with diarrhea and 202 control subjects	192
Table (10)	Grouping of the 396 diarrheal cases according to the results of direct microscopy	193
Table (11)	Results of specific ELISA for detection of <i>Entamoeba histolytica</i> , <i>Giardia intestinalis</i> and <i>Cryptosporidium</i> spp.	198
Table (13)	Comparative evaluation of direct microscopy and <i>GIARDIA</i> II ELISA test for diagnosis of <i>Giardia intestinalis</i> among cases	200
Table (12)	Comparative evaluation of direct microscopy and <i>CRYPTOSPORIDIUM</i> II ELISA test for diagnosis of <i>Cryptosporidium</i> spp. among cases	200

Table (14)	Results of Multiplex- real time PCR for detection of <i>Entamoeba dispar</i> , <i>Entamoeba histolytica</i> , <i>Giardia intestinalis</i> and <i>Cryptosporidium</i> spp.	203
Table (15)	Relation between the frequencies of multiplex- real time PCR Ct values and microscopy results of <i>Giardia intestinalis</i> among diarrheal cases	207
Table (16)	Relation between the frequencies of multiplex- real time PCR Ct values and microscopy results of <i>Cryptosporidium</i> spp. among diarrheal cases	208
Table (17)	Relation between the frequencies of multiplex- real time PCR Ct values and microscopy results of <i>Entamoeba dispar</i> among diarrheal cases	209
Table (18)	Comparative evaluation of microscopy, multiplex- real time PCR and ELISA for the detection of <i>Giardia intestinalis</i> against an expanded gold standard	211
Table (19)	Comparative evaluation of microscopy, multiplex- real time PCR and ELISA for the detection of <i>Cryptosporidium</i> spp. against an expanded gold standard	212
Table (20)	Sensitivity, specificity, positive and negative predictive values of microscopy, ELISA and PCR in the detection of <i>Giardia intestinalis</i> and <i>Cryptosporidium</i> spp. against an expanded gold standard	213
Table (21)	Comparative evaluation of direct microscopy and real-time multiplex PCR for diagnosis of <i>Entamoeba dispar</i> among cases	217
Table (22)	Comparative evaluation of direct microscopy, <i>GIARDIA II</i> ELISA and real-time multiplex PCR for diagnosis of <i>Giardia intestinalis</i> among cases	217
Table (23)	Comparative evaluation of direct microscopy, <i>CRYPTOSPORIDIUM II</i> ELISA and real-time PCR for diagnosis of <i>Cryptosporidium</i> spp. among cases	218

Table (24)	Sensitivity, specificity, positive and negative predictive values of microscopy compared to multiplex real-time PCR for detection of <i>Entamoeba histolytica/dispar</i> , <i>Giardia intestinalis</i> and <i>Cryptosporidium</i> spp. in the stool of 396 diarrheal patients	220
Table (25)	Sensitivity, specificity, positive and negative predictive values of ELISA compared to multiplex real-time PCR for detection of <i>Entamoeba histolytica</i> , <i>Giardia intestinalis</i> and <i>Cryptosporidium</i> spp. in the stool of 396 diarrheal patients	222
Table (26)	Comparative evaluation of direct microscopy, <i>GIARDIA II</i> ELISA and real-time multiplex PCR for diagnosis of <i>Giardia intestinalis</i> among controls	224

List of Figures

Figure		Page
Figure (1)	Proportional distribution of diarrhea cases among children less than five years of age, by region, 2004	12
Figure (2)	Mortality rate of children under five (per 1,000 births)	12
Figure (3)	Schematic diagram showing <i>Giardia intestinalis</i> trophozoite	22
Figure (4)	Schematic diagram for the <i>Giardia intestinalis</i> cyst	23
Figure (5)	Transmission electron microscopy (TEM) view of <i>Giardia intestinalis</i> trophozoite	23
Figure (6)	Life cycle of <i>Giardia intestinalis</i>	26
Figure (7)	Mucosal surface of the small intestine of a gerbil infected with <i>Giardia</i> protozoa	28
Figure (8)	The ultrastructural morphology of a <i>Giardia</i> protozoan's ventral adhesive disk	29
Figure (9)	<i>Cryptosporidium</i> oocyst morphology	37
Figure (10)	Transition electron microscope diagram of sporozoite	37
Figure (11)	Schematic representation of the <i>Cryptosporidium</i> spp. life cycle	41
Figure (12)	Pathogenesis of cryptosporidial enteropathy and cholangiopathy	45
Figure (13)	Schematic diagram showing trophozoite and cyst of <i>Entamoeba histolytica</i>	54
Figure (14)	Variation in nuclear structure of <i>Entamoeba histolytica</i>	55
Figure (15)	Ultrastructure of <i>Entamoeba histolytica</i> trophozoites	56
Figure (16)	Life cycle of <i>Entamoeba histolytica</i>	57

Figure (17)	Model for <i>E. histolytica</i> tissue damage in amoebic colitis	63
Figure (18)	Morphological features of <i>Entamoeba histolytica</i>	70
Figure (19)	Morphological features of <i>Giardia intestinalis</i>	73
Figure (20)	Stained <i>Cryptosporidium</i> spp. oocysts	75
Figure (21)	IFA <i>Giardia</i> and <i>Cryptosporidium</i>	77
Figure (22)	Schematic diagram of Ag detection immunocapture sandwich ELISA	79
Figure (23)	Model for Immunochromatographic test	81
Figure (24)	Principle of Immunochromatographic test	81
Figure (25)	Polymerase Chain Reaction (PCR)	102
Figure (26)	Real-time PCR	109
Figure (27)	SYBR Green I assay	110
Figure (28)	TaqMan 5' nuclease hydrolysis probe	118
Figure (29)	Real-time FRET probe technologies	119
Figure (30)	Principle of Scorpion probe technology	121
Figure (31)	Optical detection system layout	122
Figure (32)	Informed consent	140
Figure (33)	Formal ether sedimentation concentration technique, after centrifugation	144
Figure (34)	QIAamp DNA Mini stool Kit procedure	167
Figure (35)	Mechanism of PCR cycling inside Rotor gene	179
Figure (36)	Log plot of amplification curves, comparing baseline, threshold, and threshold cycle (Ct) values	181
Figure (37)	Relation between initial template amount and Ct value	183

Figure (38)	Clinical manifestations among cases	188
Figure (39)	Stool consistency among cases	189
Figure (40)	Iodine stained stool smear of a diarrheal case showing two-nucleated <i>Entamoeba histolytica/dispar</i> cyst with chromatoid bar (X 1000)	193
Figure (41)	Iodine stained stool smear of a diarrheal case showing <i>Giardia intestinalis</i> trophozoite (X 1000)	194
Figure (42)	Iodine stained stool smear of a diarrheal case showing <i>Giardia intestinalis</i> cyst (X 1000)	194
Figure (43)	A modified Ziehl Neelsen stained smear of a diarrheal case, showing <i>Cryptosporidium</i> oocyst (X 1000)	195
Figure (44)	Other parasites detected in the stool smear of diarrheal cases	195
Figure (45)	Real time PCR amplification curves for detection of <i>Giardia intestinalis</i>	204
Figure (46)	Real time PCR amplification curves for detection of <i>Cryptosporidium</i> spp.	204
Figure (47)	Real time PCR amplification curves for detection of <i>Entamoeba dispar</i>	205
Figure (48)	Real time PCR amplification curves for the internal control	205
Figure (49)	Relation between the frequencies of multiplex-real time PCR Ct values and microscopy results of <i>Giardia intestinalis</i> among diarrheal cases	207
Figure (50)	Relation between the frequencies of multiplex-real time PCR Ct values and microscopy results of <i>Cryptosporidium</i> spp. among diarrheal cases	208
Figure (51)	Relation between the frequencies of multiplex-real time PCR Ct values and microscopy results of <i>Entamoeba dispar</i> among diarrheal cases	209

Figure (52)	Sensitivity of microscopy, ELISA and PCR in the detection of <i>Giardia intestinalis</i> and <i>Cryptosporidium</i> spp. against an expanded gold standard	213
Figure (53)	Specificity of microscopy, ELISA and PCR in the detection of <i>Giardia intestinalis</i> and <i>Cryptosporidium</i> spp. against an expanded gold standard	214
Figure (54)	Positive predictive values of microscopy, ELISA and PCR in the detection of <i>Giardia intestinalis</i> and <i>Cryptosporidium</i> spp. against an expanded gold standard	214
Figure (55)	Negative predictive values of microscopy, ELISA and PCR in the detection of <i>Giardia intestinalis</i> and <i>Cryptosporidium</i> spp. against an expanded gold standard	215
Figure (56)	Sensitivity, specificity, positive and negative predictive values of microscopy compared to multiplex real-time PCR for detection of <i>Entamoeba histolytica/dispar</i> , <i>Giardia intestinalis</i> and <i>Cryptosporidium</i> spp. in the stool of 396 diarrheal patients	221
Figure (57)	Sensitivity, specificity, positive and negative predictive values of ELISA compared to multiplex real-time PCR for detection of <i>Entamoeba histolytica</i> , <i>Giardia intestinalis</i> and <i>Cryptosporidium</i> spp. in the stool of 396 diarrheal patients	223

List of contents

Abstract	I
List of Abbreviations.....	II
List of Tables.....	IV
List of Figures.....	VII
Introduction	1
Aim and Plan of the work	7
Review of literature.....	
- Diarrhea	9
- Common protozoan parasites causing diarrhea	16
- Diagnosis of enteric protozoa	64
- Real time PCR	99
Subjects and Methods	137
Results	186
Discussion	225
Conclusion	246
Recommendations and Future studies.....	247
Summary	248
References	254
Arabic summary	

