



### PRELIMINARY STUDY FOR DETECTION OF CAPILLARIA PHILIPPINENSIS COPRO-DNA IN DIARRHEIC PATIENTS

#### **Thesis**

Submitted for partial fulfillment of M.D. degree in Medical Parasitology

### By Mona Ibrahim Ali

(M.B., B.Ch. M.Sc.)
Assistant lecturer of Medical Parasitology
Faculty of Medicine/Beni Suief University

#### **SUPERVISORS**

#### Prof. Dr. Nadia Aly Essa El-Dib

Professor of Medical Parasitology Faculty of Medicine Cairo University

#### Prof. Dr. Ayman Abdel-Moamen Al-Badry

Professor of Medical Parasitology Faculty of Medicine Cairo University

#### Dr. Marwa Ahmed Ghieth

Lecturer of Medical Parasitology Faculty of Medicine Beni suief University

# In collaboration with: Prof. Dr. Jose Miguel Rubio Muňez

Professor of Parasitology
Malaria & Emerging Parasites Diseases Lab., Parasitology Department,
Microbiology Center, Instituto de Salud Carlos III, Madrid, Spain

Faculty of medicine Cairo University 2014

## Dedication

To everyone who has lent me his helping hand from my start till now.

With all my respect and appreciation.

Mona Ibrahim Ali

## Acknowledgement

First and foremost thanks are due to **Allah**, the most beneficent and merciful for helping me getting this work done.

I am so grateful to **Prof. Dr. Mona Mahmoud**, head of the Parasitology, Faculty of Medicine, Cairo University, for her help.

I would like to express my deep thanks to **Prof. Dr. Nadia Aly Eissa El-Dib,** Professor of Parasitology, Faculty of Medicine, Cairo University, for her valuable guidance, encouragement, help, advice, and overwhelming kindness. I wish to express to her my extreme gratitude.

My deepest appreciation goes to **Prof. Dr. Ayman Abdel-moamen Al-Badry**, Professor of Parasitology, Faculty of Medicine, Cairo University, for his help, extremely valuable guidance, advice, endless support, knowledge and valuable effort throughout the work. I consider an honor to work with him.

I feel great gratitude to **Dr. Marwa Ahmed Ghieth,** Lecturer of Parasitology, Faculty of Medicine, Beni suief University, for her cooperation, encouragement, and help.

I wish to express my extreme gratitude to **Prof. Dr. Jose Meguil Rubio Muňez,** Professor of Parasitology, Malaria & Emerging Parasites Diseases Lab., Parasitology Department, Microbiology Center, Instituto de Salud Carlos III, Madrid, Spain, for his part in designing the work, kind supervision, help, and cooperation.

I would like also to express my thanks to the staff members of Internal, Tropical, and Clinical Pathology Medicine Departments of Faculty of Medicine, Beni-Suief University for their help in detection and collection of the clinical data of cases.

I am really thankfull to all my Professors & colleagues in Medical Parasitology Department, Beni suef University and Cairo University for their ever encouragement to complete this work.

Finally, I would like to express my warm feelings to all my family members, So, I take this opportunity to thank them especially my parents, my daughters and my husband.

## **Table of contents**

List of Abbreviations	i
List of tables	iii
List of figures	v
Abstract	viii
1. Introduction	1
2. Aim of the work	4
3. Review of literature:	
3.1. Classification	5
3.2. Prevalence	6
3.3. Transmission	8
3.4. Life cycle	9
3.5. Morphology	12
3.6. Pathogenicity and clinical picture	15
3.7. Diagnosis	18
3.8. Differential diagnosis	32
3.9. Treatment	34
3.10. Prevention and control	36
4. Materials and methods	37
5. Results	61
6. Discussion	87
7. Summary	106
8. Conclusion 1	09

9. Recommendation	111
10. References	. 112
11. Appendix	130
ربی	الملخص العر

## List of abbreviations

Вр	Base pair
CI	Confidence interval
CT	Computerized tomography
C. aerophila	Capillaria aerophila.
C. boehmi	Capillaria boehmi
C. hepatica	Capillaria hepatica.
C.obsignata	Capillaria obsignata
C. philippinensis	Capillaria philippinensis.
C. putorii	Capillaria putorii
cox1	cytochrome c oxidase subunit 1
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
ddH <sub>2</sub> O	Distilled water
ELISA	Enzyme linked immunosorbent assay.
Hb	Hemoglobin.
IHA	Indirect hemagglutination
Ig	Immunoglobulin
Lao PDR	Lao People's Democratic Republic.
mtDNA	Mitochondrial DNA
nPCR	Nested polymerase chain reaction
NPV	Negative predictive value
OR	Odds ratio
PPV	Positive predictive value
RBCs	Red blood cells
Rx	Master mixture

SD	Standard deviation
TBE	Tris boric acid EDTA
$T_{ m m}$	Melting temperature
T. vulpis	Trichuris vulpis
18S rRNA	small subunit ribosomal RNA

## List of tables

Title	Page No.
Table 4.1: The used primers and their sequences.	52
Table 4.2: The optimized 1ry and 2ry PCR assays reaction components.	54
Table 4.3: Thermocycler program showing the used cycling conditions in	55
1ry PCR.	
Table 4.4: Thermocycler program showing the used cycling conditions in nPCR.	56
Table 5.1: Mean age of studied individuals.	62
Table 5.2: Distribution of age groups and gender among study individuals.	63
Table 5.3: Frequency of different complaints of the study group.	64
Table 5.4: Mean diarrhea duration and number of motions of studied individuals.	64
Table 5.5: Clinical manifestations of study group.	65
Table 5.6: Yield of macroscopic examination of stool collected from studied	66
individuals.	
Table 5.7: Results of microscopic examination of faecal samples of study group.	71
Table 5.8: Diagnostic yield of microscopy and nPCR in detection of <i>C</i> .	72
philippinensis among both studied groups.	
Table 5.9: Diagnostic yield, accuracy and Kappa agreement of the microscopy	75
method among study individuals.	
Table 5.10: Mean age of <i>C. philippinensis</i> cases in the study group.	77
Table 5.11: Demographic and environmental data of nPCR positive cases for <i>C</i> .	78
philippinensis.	
Table 5.12: Distribution of diarrhea duration and motions among	80
C.philippinensis positive cases.	
Table 5.13: Associated clinical manifestations among <i>C. philippinensis</i> positive	81
cases.	

Table 5.14: Yield of macroscopic examination of stool collected from	82
C.philippinensis positive cases.	
Table 5.15: Results of stool examination of positive <i>C. philippinensis</i> cases.	83
Table 5.16: Data analysis for nPCR <i>C. philippinensis</i> positive cases.	85
Table 5.17: Clinical manifestations analysis for nPCR <i>C. philippinensis</i> positive	86
cases.	

# List of figures

Title	Page No.
Figure 3.1: Proposed life cycle of <i>C. philippinensis</i>	11
http://dpd.cdc.gov/dpdx/HTML/ intestinal capillariasis.htm	
Figure 3.2: A, SEM showing a striated cuticle both transversely and	13
longitudinally and a small zigzag cuticular expansion. B, SEM showing a	
wide, long cleft on the undersurface of the body of a worm (Attia et al.,	
2012).	
Figure 3.3: <i>Capillaria philippinensis</i> , adult female showing narrow	13
anterior and wider posterior portions. Note stichosome (thick arrow) and	
eggs in the uterus (thin arrow) (Cross, 1997).	
Figure 3.4: Adult male <i>C. philippinensis</i> . Thick arrow refers to the spicule	14
while thin one refers to the sheath (Amin et al., 2011) ( $\times$ 100).	
Figure 3.5: A mature egg, having characteristic mucoid plugs at both ends	14
(Jung et al., 2012).	
Figure 3. 6: Larva of <i>C. philippinensis</i> (El- Dib <i>et al</i> 1999) (x 1000).	15
Figure 3.7: Photomicrograph of the biopsy specimen from the distal ileum	22
(H&E stain, ×200).It show atrophic changes of the intestinal villi,	
infiltration of plasma cells and eosinophils, and several sections of worms	
invading the mucosa (arrows) (Ha et al., 2013).	
Figure 3.8: Abdominal radiograph. Small bowel series performed with	26
barium illustrates marked luminal narrowing and effacement of folds	
producing a ribbon-like appearance of the ileum (Ha et al., 2013).	
Figure 4.1.A: Area for nuclic acid extraction.	42
Figure 4.1.B: The area for preparing nPCR and gel electrophoresis.	43

Figure 4.2: Favor Prep stool DNA isolation Mini Kit.	46
Figure 4.3: DNA extraction by Favor Prep stool DNA isolation Mini Kit.	49
Figure 4.4: Showing laminar flow cabinet.	51
Figure 4.5: Thermal cycler used in DNA amplification.	51
Figure 4.6: Showing gel electrophoresis apparatus.	57
Figure 5.1: Age groups among the study group.	63
Figure 5.2: Eggs of <i>C.philippinensis</i> ( $\times$ 100).	67
Figure 5.3: Peanut-shaped egg of <i>C. philippinensis</i> with flattened bipolar	67
mucoid plugs and shell striations ( $\times$ 400).	
Figure 5.4: Eggs of <i>C. philippinensis</i> with Charcot Leyden crystals (×	68
400).	
Figure 5.5: Adult female <i>C.philippinensis</i> showing eggs in the uterus (the	68
arrows) (× 100).	
Figure 5.6: Posterior end of an adult male <i>C.philippinensis</i> showing the	69
spicule (× 400).	
Figure 5.7: Charcot Leyden crystals (× 400).	69
Figure 5.8: Diagnostic yield of microscopy for detection of	73
C.philippinensis compared with nPCR of study group.	
Figure 5.9: Showing Agarose gel electrophoresis for the products of the	74
PCR targeting CAP 18S gene of <i>C. philippinensis</i> at 183 bp.	
Figure 5.10: Results of blood chemistry of microscopiv <i>C. philippinensis</i>	76
positive patients.	
Figure 5.11: Mean age of nPCR positive and negative cases in the study	78
group.	
Figure 5.12: Age distribution of nPCR positive cases in <i>C. philippinensis</i>	79
infected cases.	
Figure 5.13: Gender distribution of nPCR positive cases among	79
C.philippinensis positive cases.	

Figure 5.14: Associated clinical manifestations among <i>C.philippinensis</i>	81
positive cases.	
Figure 5.15: Combined infection of both <i>C.philippinensis</i> and	84
schistosoma mansoni eggs (x100).	

#### **Abstract**

(**Key words**: *Capillaria philippinensis*, Intestinal capillariasis, Chronic diarrhea, Nested PCR, *Capillaria* Molecular diagnosis).

C. philippinensis is a parasite of the small intestine that causes a severe enteropathy and at times death in cases of delayed diagnosis or inappropriate treatment. Molecular-based approaches offer greater sensitivity and specificity over the existing diagnostic tests and it can help in the diagnosis of Capillaria infected cases. The aim of our study is to develop a molecular diagnostic method, by utilization of PCR-based assay for detection of *C. philippinensis*. This study was conducted over 250 patients attending the outpatient clinics of Beni-Suief University hospital. Stool samples were collected and subjected to coproparasitological examination and copro- nested PCR (nPCR) assays. By microscopic examination, *C. philippinensis* was detected in stool samples of 36 (24%) individuals, all were complaining of chronic diarrhea. By nPCR assay, Copro-DNA was detected in the fecal samples of these 36 positive cases and in fecal samples of other seven individuals whose stool samples were negative for *Capillaria* by microscopic examination. *C*. philippinensis-specific PCR method has been successfully used and evaluated in this study. It is a rapid specific and accurate test that identifies infection with this parasite avoiding the delay in management and possible complications

## 1. Introduction

Human intestinal capillariasis is a parasitic disease caused by *Capillaria philippinensis*, a nematode parasite of fish-eating birds. Although man is accidentally infected by this parasitic disease, its outcome may be fatal if untreated in due time (Soukhathammavong *et al.*, 2008 and Attia *et al.*, 2012).

*C. philippinensis*, first documented in the Philippines in 1963 by Chitwood *et al.* (1964) Subsequently, it has been reported in the Philippines and Thailand, and sporadic cases of the disease were diagnosed in other areas (Austin *et al.*, 1999). In Egypt, Yousef et al., 1989, reported the first case.

Intestinal capillariasis is a life-threatening disease in humans that causes severe enteropathy (Cross, 1998). The most common pathological features are the thickening of the intestinal wall, the deepening of the crypts, and the atrophy of the villi leading to malabsorption of all nutrients (Sangcha *et al.*, 2007). The infestation of the small bowel by this parasite leads to weight loss, chronic diarrhea, abdominal pain, borborygmi, muscle wasting, cachexia, weakness, edema, ascites and/or pleural effusion as a complication of hypo-albuminaemia. Death may