

بسم الله الرحمن الرحيم





شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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Cairo University



The role of horses and donkeys in emerging of Cryptococcosis in Egypt

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For

The Degree of M.V.Sc

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Abstract

Cryptococcus has gained medical importance over the last decade, as it represents a potential risk for immunosuppressed and immunocompetent individuals. Previous studies reported cryptococcosis in equines is uncommon, sporadic cases have been reported with rhinitis, sinusitis, pneumonia and meningitis. There are no epidemiological data on the prevalence of this fungus in horses and donkeys. The current study was carried to investigate the possible role of these animals in the epidemiology of such pathogen. A total of 275 samples were collected from different localities in Egypt included 263 nasal swabs (183 from horses, 52 from donkeys and 28 from humans) in addition to 12 soil samples. Bacteriological examination and identification of *Cryptococcus* were performed. The overall occurrence of *Cryptococcus* spp. in the investigated samples was 14.8, 11.5, 25 and 10.7% in horse, donkeys, soil and human, respectively. Molecular serotyping of *Cryptococcus* spp. isolates recovered from the nasal passage of horses and donkeys proved that *C. gattii* (B) and *C. neoformans* were identified; in addition, two hybrids between *C. neoformans* (A) and *C. gattii* (B) were identified in horse samples. While in case of soil samples, the isolates were identified as *C. gattii* (B). The human isolates were serotyped as *C. gattii* in two isolates and *C. neoformans* in only one isolate. Molecular detection of some virulence genes among the identified isolates showed that *LAC1* and *CAP59* genes have been the most frequently detected genes. In horse, soil and human samples both *LAC1* & *CAP59* genes were identified in *C. gattii* and *C. neoformans* isolates, on the other hand *PLB1* detected only in *C. gattii*. While the identified virulence genes in donkeys' *C. gattii* and *C. neoformans* isolates demonstrated higher laccase (*LAC1*) genes. While capsular associated protein (*CAP59*) gene identified alone or associated with *LAC1* gene in *C. gattii* isolates. Phylogenetic analysis of *C. gattii* from horses, human and soil samples that found in close vicinity demonstrated that they were closely related. To our best knowledge this is the first research evaluating *Cryptococcus* species emergence in equines and donkeys. This study provides the first insights into the ecology of *Cryptococcus* species in Egypt and highlights the role of these animals as asymptomatic carriers for disseminating the potentially pathogenic *Cryptococcus* spp in the environment. It also reflects the potential risk of human infection with cryptococcosis. In order to strengthen existing therapeutic and control approaches, further analyses of the main risk factors and the other virulence of these pathogens should be considered too.

Keywords: Horse, Donkeys, soil, human, *C. neoformans*, *C. gattii*, nasal swabs, serotyping, virulence factors.

Dedication

I would like to dedicate this work to my father, my mother and sisters, whom I am indebted to them for my happiness in my life.

Acknowledgments

*First of all, I would like to express my prayerful gratitude and great thanks to the merciful **ALLAH** whose help me always seek, and without his will, I shall achieve nothing.*

*I wish to present my respect, deepest gratitude and highest appreciation to **Prof.Dr. Maha Ahmed Sabry** professor of zoonoses, Faculty of Veterinary Medicine, Cairo University for planning this research program, sincere supervision, wise scientific advices and encouragement during this study which made this thesis in the best way.*

*I am extremely grateful to **Dr.Dalia Anwar Hamza** Assistant professor of zoonoses, Faculty of Veterinary Medicine, Cairo University for her valuable advices, continuous encouragement and constructive criticism. She offered continuous help and support.*

*I would like to express my appreciation to **Dr. Sara M. Nader** lecturer of zoonoses, Faculty of Veterinary Medicine, Cairo University for her valuable help, everlasting advice, kind support and continuous encouragement.*

I would like to thank all staff members of the department of zoonoses for their encouragement and kind support during this work,

*No words enough to thank my lovely friends **Fatma Karam, Sabah Ali, Asmaa Rabiee** for their continuous support in my hard time.*

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List of abbreviations

Abbreviation	Full name
AIDS	Acquired Immune Deficiency Syndrome
BLAST	Basic Local Alignment Search Tool
Bp	Base pair
<i>C.deneoformans</i>	<i>Cryptococcus deneoformans</i>
<i>C.deuteroformans</i>	<i>Cryptococcus deuteroformans</i>
<i>C.gattii</i>	<i>Cryptococcus gattii</i>
<i>C.hominis</i>	<i>Cryptococcus hominis</i>
<i>C.neoformans</i>	<i>Cryptococcus neoformans</i>
CAP	Capsule associated protein
CDC	Centers for Disease Control and Prevention
CGB	Canavanine Glycine-Bromothymol blue
CLUSTALW	Clustal alignment format without base / residue numbering
CNS	Central nervous system
<i>Cryptococcus spp.</i>	<i>Cryptococcus</i> species
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
E.C	Eucalyptus Camaldulensis
FAO	Food and Agriculture Organization

Abbreviation	Full name
Fig	Figure
G	Gram
g/L	Gram/liter
GALXM	Galactoxylomannan
GXM	Glucuronoxylopolysaccharide
HIV	Human Immunodeficiency Virus
IACUC	Institutional Animal Care and Use Committee
ICU	Intensive care unit
L.DOPA	levodopa dihydroxyphenylalanine
LAC	Laccase
ml	milliliter
MP	Mannoproteins
μl	Microliter
μM	Micromole
NAMRU3	Naval Medical Research Unit No 3
NCBI	National center for Biotechnology information
PASW	Predictive Analytics Software (applied statistical software)
PCR	Polymerase chain reaction
PLB	Phospholipase

Abbreviation	Full name
Pmole/ µl	Picomoles / µl
RYP	RapID yeast plus system
S.homnis	Saccharomycosis hominis
<i>S.neoformans</i>	Saccharomyces neoformans
SDA	Sabouraud dextrose agar
SOT	Solid organ transplantation
SPSS	Statistical Package for the Social Sciences
<i>T.histolytica</i>	Toula histolytica
TAM	Tobacoo agar media
Taq	Thermus aquaticus
USA	United States of America
V	Volt
Var	Variety