

Deanship of Graduate Studies

Al-Quds University



**Identification of Microbial Community in Rock Hyrax,
Suspected Reservoir of *Leishmania tropica* in Palestine: A
Metagenomic Analysis**

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M.Sc. Thesis

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Prepared By: Rana Nayef Mesleh Always

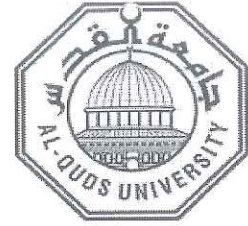
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Al-Quds University
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Thesis Approval

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of *Leishmania tropica* in Palestine: A Metagenomic Analysis**

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**Jerusalem-Palestine
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Jerusalem – Palestine

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Dedication

For

The sake of Allah, my Creator and my Master, who always inspires me how to believe in myself and my dreams.

My great teacher and messenger, Mohammed (May peace be upon him), who taught us the purpose of life.

My homeland Palestine and Jerusalem, the city of peace.

To those dearest to me, my family, my mother and my father, who offered me unconditional love constant and source of support and encouragement during the challenges of life.

To my brothers and their kids, who draw a smile and laugh on my face every day, my sister, my unconditional special friends and all people who have always been there to support me

To my role model in life, my uncle Mohammad Al Shab'aan, the petroleum geologist

To my friends and colleagues.

I dedicate this research.

Rana Nayef Mesleh Alwaysa

Declaration

I certify that this thesis submitted for the Degree of Master is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

Signed.....

Rana Nayef Mesleh Awaysa

Date: 11.1.2020

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Abstract

Hyrax (*Procavia capensis*) is the only representative of the order Hyracoidea in the Middle East. This African species harbor *Leishmania* in their skin and nematodes in their gut, they are susceptible to viral pneumonia and tuberculosis. The widespread co-distribution of this reservoir, suggests a significant threat from the spread of diseases caused by pathogens accompanying with the body of hyraxes. Hyraxes can live several years in nature and thus comprise a natural reservoir for carrying and transmitting infections. Several studies showed that experimentally infected hyraxes by *Leishmania* can sustain infection, showing no clinical signs but they are infective to sand flies. Therefore, this study aimed to investigate the microbial community structure of tissue samples taken from hyraxes collected from different regions in Palestine which may pose a threat to human health.

Sixteen rock hyraxes have been collected using raccoon traps from different localities in Palestine (A'nabta-Tulkarem, Haifa, Amara near Beer Al-Saba'a, Mas'ha-Salfit and Faqqua-Jenin). The DNA was extracted from hyraxes tissue samples (n=16) including blood (n=3), nose (n=2), ear (n=3) and spleen (n=8), only one sample was taken from each hyrax. All DNA samples were tested for presence of pathogens including parasites, bacteria, viruses and fungi using next generation sequencing (NGS). DNA library were prepared based on Nextera® XT assay and deep sequenced on Illumina MiSeq Machine. Bioinformatics analysis was applied to identify the NGS obtained sequences using the online tool on Galaxy . The retrieved pathogenic sequences were blasted using BLAST search on NCBI . The sequences with >97% identity and >97% query cover were adopted and compared to the available reference nucleotide sequences in the database for species identification.

Among the tested hyraxes samples (n=16), four samples including spleen (n=2), blood (n=1) and nasal tissue (n=1) were excluded from the study. Therefore, the analysis was applied only on twelve hyrax samples, from which *Leishmania* have been detected in four hyrax samples, which were obtained from spleen (n=3) and ear (n=1). Three of them were identified as *L. tropica* while the forth sample was identified only up to the genus level . These hyraxes were collected from A'nabta-Tulkarem and Haifa where cases of human cutaneous leishmanias due to *L. tropica* were reported. Furthermore, at the bacterial level, *Mycobacterium tuberculosis complex* (MTC) has been detected in three hyraxes. These hyraxes were captured from Haifa,

Faqqua-Jenin and Amara near Beer Al-Saba'a. On the other hand, human pathogen such as, *Neisseria meningitides* have been also detected in tow hyraxes in addition to *Bordetella spp.* which was detected in one hyrax. Moreover, two types of viruses in three hyrax samples, the two dominant viruses were *Human betaherpesvirus* and *Procavia capensis gammaherpesvirus*.

In conclusion, our results showed that rock hyraxes can be involved in transmission of serious pathogens which can be harmful to humans as hyraxes have long lifespan and gregarious habits sharing habitats with several vectors. Our results confirmed the presence of *L. tropica* DNA in rock hyraxes from Palestine and supported their potential role as a reservoir for human CL.. Efforts to prevent human leishmaniasis should be focused on interrupting the transmission of infection cycle that includes in addition to the control of sandfly vectors the control of hyrax population as a reservoir.

Key words: **Key words:** Hyrax, reservoir, *Leishmania*, Cutaneous leishmaniasis, *Mycobacterium*, *Human betaherpesvirus*, metagenomics, Galaxy.

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List of Abbreviation

Abbreviation Full Term

CL	Cutaneous Leishmaniasis
WHO	World Health Organization
MAP	<i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i>
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
kDNA PCR	kinetoplast DNA- polymerase chain reaction
nPCR	nested polymerase chain reaction
NGS	Next-generation sequencing
EDTA	Ethylenediaminetetraacetic acid
TE	Tris-EDTA
NT	Nucleotide
WGS	Whole Genome Sequencing
BLAST	Basic Local Alignment Search Tool
BLASTN	Nucleotide BLAST
NCBI	National Centre for Biotechnology Information
SNP	Single Nucleotide Polymorphisms
MTC	<i>Mycobacterium tuberculosis complex</i>

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Chapter One: Introduction

1.1 Background:

Hyrax (*Procavia capensis*) is the only representative species of the order Hyracoidea in the Middle East. Rock hyrax is mainly distributed in Africa and the Middle East. In Palestine, the rock hyrax has been reported on the mountains of the western side of the Dead Sea and the Jordan Valley, including Wadi Qelt, west of Jericho city and Zubaidat village 50 kms north of Jericho city. The presence also extends to the northern Palestinian districts of Tubas and Jenin on the outskirts of villages close to human activity and even reaching the Lake of Tiberias with populations also found close to Umm Al-Rashrash (Al-Jawabreh et al., 2017; Talmi-Frank et al., 2010). Rock hyraxes harbor *Leishmania* in their skin and nematodes in their gut; they are susceptible to viral pneumonia and tuberculosis. Furthermore different ecto-parasites also infect hyraxes (Lemma, 2008; Rifai et al., 2000). The widespread distribution of this reservoir, suggests a significant threat from the spread of diseases caused by pathogens accompanying with the body of hyraxes in the Middle East, central Asia and southern Europe (Faiman et al., 2013).

1.2 Classification:

The hyraxes, from the Greek word ‘hyrak’ meaning shrew-mouse, are classified under order hyracoidean, the order Hyracoide consists of one extant family, Procaviidae and one extinct family, Pliohyracidae. The extant family comprises three living genera; *Procavia*, *Heterohyrax* and *Dendrohyrax*. Members of the genera *Procavia* Storr are strictly rock-dwelling and considered to have evolved first in Africa before the Oligocene period 40 million years ago (Teklehaimanot and Balakrishnan, 2018; Walker et al., 1975). *Heterohyrax* Gray is rock-dwelling and partially arboreal bush hyrax, whereas *Dendrohyrax* Gray is strictly arboreal (Stuart and Stuart, 2007; Teklehaimanot and Balakrishnan, 2018), (Figure 1.1). Hyrax belongs to super order Afrotheria which includes elephants, sea cow, elephant shrew, aardvark and dugongs (Springer et al., 1997). Hyraxes and elephants share many of the characteristics of the nature karyotype, this supports the development of African animal that group within the superorder of Paenungulata (Al-Dakan and Al-Saleh, 2015; Pardini et al., 2007).