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Ecdysone receptor knockdown: implications on longevity, reproductive success and plasmodium susceptibility in the malaria vector anopheles funestus

Maharaj, Surina

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Ecdysone Receptor Knockdown: Implications on Longevity, Reproductive Success and *Plasmodium* Susceptibility in the Malaria Vector *Anopheles funestus*.

Surina Maharaj

A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science in Medicine.

Johannesburg, June 2021

Declaration

I, Surina Maharaj declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Masters of Science at the University of Witwatersrand, Johannesburg.

It has not been submitted before for any degree or examination at any other University.



Surina Maharaj

28 day of June 2021 in Johannesburg

Abstract

Malaria is a devastating disease in humans, transmitted by female *Anopheles* mosquitoes infected with *Plasmodium* parasites. Current insecticide-based strategies exist to control the spread of malaria by targeting vectors. Unfortunately, the effectiveness of these vector control methods is declining due to increasing insecticide resistance in vector populations. It is therefore essential to develop novel vector control methods that can efficiently target transmission reducing factors such as vector density (influenced by vector survival and reproduction) and competence (influenced by transmission ability). A possible vector control candidate gene, the ecdysone receptor (EcR) regulates longevity, reproduction, immunity, as well as many other physiological processes in several insects and malaria vectors. The malaria vector, *Anopheles funestus* (*An. funestus*) is responsible for the majority of malaria outbreaks in South Africa, however, the function of *EcR* in this vector has not previously been studied. This study therefore aimed to determine the extent of *EcR* regulation on longevity, reproduction and susceptibility to *Plasmodium falciparum* (*P. falciparum*) in *An. funestus*. In this study, RNA interference was used to reduce *EcR* expression levels in *An. funestus* females and investigate how it impacted their longevity, reproduction and susceptibility to *P. falciparum* infection. Additionally, the expression levels of *EcR*, and reproduction genes lipophorin (*Lp*) and vitellogenin receptor (*VgR*) as well as the immune gene, leucine rich immune molecule 9 (*LRIM9*) were determined using qPCR and relative expression analysis was performed. *EcR*-depleted mosquitoes had a shorter lifespan, were less fecund, less fertile, and had reduced *P. falciparum* infection intensity. Moreover, gene expression analyses revealed that mosquitoes with reduced *EcR* expression also had depleted *Lp, VgR* and *LRIM9* expression levels. As EcR regulates processes affecting vector density and competence, this study provides the first experimental evidence that supports EcR as a novel target in the development of vector control measures targeting *An. funestus*.

Ethics waiver

ANIMAL RESEARCH ETHICS COMMITTEE

Registration number: AREC-101210-002

Date: July 1, 2019

Certificate reference: 20190701-7O
Category: O
Re: Waiver from the Animal Research Ethics Committee of the University of the Witwatersran

Reference : Animal Ethics to complete
Re: Waiver from the Animal Research Ethics Committee of the University of the Witwatersrand
HoD applicant : Lizette Koekemoer
Staff number : 09700304
Degree : PhD/Msc Candidates
Study Title : "Studies on malaria vectors".
Department: Wits Research Institute for Malaria;
laboratory
Supervisor: Lizette Koekemoer, Givemore Munhenga, Yael Dahan-Moss, Shune Oliver, Basil Brooke and Maria Kaiser
Email: lizette.koekemoer@wits.ac.za
Address: 7 York Road, Parktown, Johannesburg, 2001
Tel: + 011-717 2486;
Full clearance certificate from the AREC of the University of the Witwatersrand is NOT required.
Reason: This study uses mosquitoes (invertebrates).
Comment/Notes :

1) This is a General Waiver for the PI and listed Supervisors.
2) Individual researchers and MSc & PhD candidates may require Ethics Clearance in the own name according to applicable rules of the Post-Graduate and other Committees.
3) Feeding of the mosquitos should be specified.

Please contact me should you require further information.

GP Candy

Geoffrey Candy PhD
Chair : Animal Research Ethics Committee

Geoffrey P Candy PhD
Professor, Department of Surgery, Faculty of Health Sciences, University of the Witwatersrand, *7 York Road Parktown 2193, Johannesburg; Tel: 27-11-717-2574; Email: geoffrey.candy@wits.ac.za*

Bioethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Research Office

INSTITUTIONAL BIOSAFETY COMMITTEE
(R 14/16)

CLEARANCE CERTIFICATE **PROTOCOL NUMBER: 20180402Lab**

BRIEF DESCRIPTION OF APPLICATION:

Laboratory situated in room 10M02 10th Floor, Wits Research Institute for Malaria, Medical School

APPLICANT: Professors M/LL Coetzee and Koekemoer

SCHOOL/DEPARTMENT : Molecular Medicine and Haematology

DATE CONSIDERED: By Circulation 29 November 2018

DECISION OF COMMITTEE: Approved unconditionally

These laboratories are considered to meet the standards of the Institutional Biosafety Committee (IBC), for BSL2 approval, as specified in the IBC's published Standard Operating procedures.
Any change to the type of biohazardous agents being handled should be reported to the IBC
DAFF - Reg no 39.2/University of Witwatersrand - 18/041 expiry date 03 December 20-21

1. This clearance certificate expires on 03 December 2023 and may be renewed on application.

DATE OF APPROVAL: 03 December 2019

CHAIRPERSON: James F. S. Larkin 2019.04.15 12:07:53 +02'00'

(Professor J Larkin)

DECLARATION OF APPLICANT:

To be completed in duplicate and **one copy** returned to the University of the Witwatersrand, Faculty of Health Sciences, Research Office, Office 301, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown,2193.

1. I have read, understood and accepted the approval conditions above
2. I note that the University Safety Officer, or his/her representative, may at any reasonable time inspect my laboratory or trial site to ensure compliance with current Health and Safety legislation. I undertake to offer my full co-operation in any such inspection.
3. I have read, understood and will comply with the *recommended standard operating procedures for the handling of biohazardous materials* posted at http://intranet.wits.ac.za/academic/uro/Pages/Institutional-Biosafety-Committee-(IBC).aspx

Signed: Koekemoer **Date:** 1/1/2019

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Certificate for registration of facility

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Genetic Resources, Department of Agriculture, Forestry and Fisheries
Private Bag X973, Pretoria 0001

Enquiries: Bathobile Mahlangu • **Tel**: 012 319 6165 • **Fax:** 012 319 6298 • **E-mail**: BathobileM@daff.gov.za •**Ref**: 39.2/University of Witwatersrand -18/019

Prof P B Arbuthnot
University of Witwatersrand
Faculty of Health Sciences
School of Pathology
Department of Molecular Medicine and Haematology
Wits Research Institute for Malaria
7 York Road
Parktown
2193

+27 (0) 11 717 2365 **(Tel)**
+27 (0) 11 717 2395/ +27 (0) 86 529 6833 **(Fax)**
Patrick.Arbuthnot@wits.ac.za **(E-mail)**

Dear Prof Arbuthnot

RE: REGISTRATION OF FACILITY

With reference to the application to register a facility, submitted in terms of the Genetically Modified Organisms Act, 1997 (Act No. 15 of 1997). Registration number **39.2/University of Witwatersrand -18/041.**

The facility is hereby registered; please find attached your certificate which serves as proof of registration. Please familiarize yourself with the Standard Operating Procedure approved for Regulation 2(2) to determine whether your current activities or any future activities would require an additional contained use permit or not.

Please consult the website of the Department at www.daff.gov.za (Branches, Agricultural Production, Health & Food Safety/ Genetic Resources/ Biosafety) for the latest application forms and the SOP document referred to in the above paragraph.

If any of the provisions of the Genetically Modified Organisms Act, 1997 (Act No. 15 of 1997), including any condition of any permit issued in terms of the GMO Act, is not complied with at all times, you will be subject to prosecution in terms of Section 21 of the GMO Act, 1997.

Yours sincerely

DIRECTORATE GENETIC RESOURCES
2018 -12- 03
PRIVATE BAG X973 PRETORIA 0001
REGISTRAR - GMO ACT

Ms N L Mkhonza
Registrar: Genetically Modified Organisms Act, 1997 (Act No. 15 of 1997)