Abstract of Master's Dissertation

No.1

Course	Health Innovation	Name	Tee Joseph
Thesis title	Development of transgenic *Leishmania major* expressing, red-shifted luciferase/mNeonGreen		

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Background: Leishmaniasis is an infectious disease affecting at least 12 million people globally and 350 million people are at risk of this infection. According to the World Health Organisation (WHO), it is one of the neglected tropical diseases caused by more than 20 *Leishmania spp.* and transmitted to mammalian hosts by sand fly vector. Ambisome, a drug used for the treatment of leishmaniasis works well for visceral leishmaniasis (VL) while Miltefosine, an expensive oral drug, treats all forms of leishmaniasis. However, the drug toxicity and resistance are considerable. New antileishmanial drugs and effective vaccines are needed to circumvent the limitations. The application of reporter genes has become an important area that enhances drug and vaccine development. Transgenic parasites expressing reporter proteins have become valuable tools for understanding the disease pathogenesis that enhances the screening of novel compounds. For this reason, a chimeric protein, expressing red-shifted luciferase and mNeonGreen (RE9H-mNeonGreen) *Leishmania major* was engineered. This strain will allow us to monitor and visualise the parasite growth both *in vitro* and *in vivo* studies.

Objective: This study focused on the development of transgenic *Leishmania major* parasites, expressing RE9H-mNeonGreen to apply the bioluminescence and fluorescence imaging technology for drug and vaccine screening.

Methods: The sequence of RE9H-mNeonGreen was codon-optimised and inserted into the PUC-GW-Kan vector. The codon-optimised RE9H-mNeonGreen was subcloned into the pLEXSY-pac2.1 expression system at the *BglII* and *Not1* restriction sites and used to transform One Shot™ TOP10 *Escherichia coli* by heat-shock. *L. major* were transfected at a logarithmic stage of the promastigotes with linear Swa1-targeting fragment obtained from pLEXSY-pac-HX1-RE9H-mNeonGreen following the Amaxa short protocol. The transfectants were characterised for their stability and viability *in vitro.*

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Results: Results indicated, a successful generation of RE9H-mNeonGreen^{+}*L.major* lines that could serve as a basis for further research to facilitate the development of new antileishmanial drugs or vaccines *in vivo* and *in vitro*. Using a microplate reader, a linear and robust positive correlation ($r = 0.96$, $p \leq 0.001$) was observed between the number of promastigotes and the light emitted *in vitro*. Fluorescence microscopy conducted on the promastigotes confirmed the successful integration of RE9H-mNeonGreen into *L. major*, as uniform green fluorescence was observed throughout the parasite's population. The limit of detection of the luciferase-expressing *L. major* strain was 10^1 promastigotes for each well, indicative of the sensitivity of the bioluminescence-based approach.

Conclusion: *L. major* promastigote lines were successfully transfected with RE9H-mNeonGreen. The parasites were observed to have acquired both fluorescent and bioluminescent properties that will allow for easy track and visualisation. In addition, these transgenic *L. major* parasites can be used in the downstream application for various studies *in vitro* and *in vivo* to facilitate the development of new drugs and vaccines.

(441 words)