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**DEVELOPMENT OF *IN VITRO* ASSAYS FOR
DETECTION OF ANTHELMINTIC RESISTANCE
IN CATTLE NEMATODES**

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2007

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IN CATTLE NEMATODES**

**A THESIS PRESENTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF**

**MASTER OF SCIENCE (ANIMAL SCIENCE)
AT MASSEY UNIVERSITY – PAMERSTORN NORTH
NEW ZEALAND**

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2007

ABSTRACT

The principle aim of the current research was to modify the larval development assay (LDA) for use with *Cooperia* from cattle. A series of experiments were conducted in order to modify the LDA protocol to determine the most appropriate culture media and incubation temperature.

These initial experiments concluded that, of the protocols examined, a culture medium of $\frac{1}{8}$ th the concentration of *E. coli* (EC) + $\frac{1}{4}$ th the concentration of yeast extract (YE) as generally used to culture sheep nematodes, at a culture temperature of 18°C, resulted in the optimum number of *Cooperia* larvae developed to the third larval stage (L₃). However, the number of eggs that developed to L₃ was still generally low. A comparison was then made using isolates from a farm with a history of resistance in *Cooperia* to ivermectin (IV) and benzimidazoles (BZ) and two farms with a history of no resistance in this parasite. These experiments were undertaken using $\frac{1}{8}$ EC + $\frac{1}{4}$ YE media protocol and $\frac{1}{2}$ EC + $\frac{1}{2}$ YE concentration of the standard culture media for sheep nematodes.

These three isolates were cultured at temperature of 18°C and 25 °C in the commercially available DrenchRite® 96-well microtitre assay plates which contained BZ, levamisole (LV) and IV in doubling dilutions within an agar matrix. The LD₅₀ values were determined from a dose response curve. The resulting LD₅₀ values were very variable, especially for the IV analogues. There was no obvious difference between the resistant and susceptible farms for the LD₅₀ values of BZ or IV. A secondary aim of this research was to investigate the potential usefulness of the larval feeding inhibition assay (LFIA).

This was adopted as published and it was determined it could be used to distinguish between susceptible and resistant *Teladorsagia circumcincta* with a resistance ratio of at least six. This research concluded that further research is required to fully optimise the LDA for *Cooperia* in cattle but adequate dose response curves were determined to indicate it struggles to distinguish BZ and IV resistance.

The LFIA deserves to be further investigated as it offers some scope to detect ivermectin resistance in cattle nematodes as the dose response curves demonstrated a good repeatability for *T. circumcincta* from sheep. Comparing LDA and LFIA, both assays seemed to be useful but the latter was considered to have greater potential.

ACKNOWLEDGEMENTS

This work would have not been completed without the help of many people. I am pleased to acknowledge the assistance I receive from distinguished people.

Thanks to my chief supervisor, Associate Professor Bill Pomroy, for your valuable guidance, patience and encouraging-constructive-criticism during the time of my study and my research. Thanks to my co-supervisor Dr. Ian Scott for your assistance, expertise, and friendly attitude (plus the above-mentioned chief supervisor's comments) during my study time.

Most sincere appreciation to Ms Adlington, B. (Barb) and Tunncliffe, A. for your enthusiastic technical assistance, helping in sample collection as well as looking after my sheep while I was away. Please don't go away for any other incoming students.

Thanks to Ms Tania Waghorn from AgResearch Centre for the donation of samples. Thank you also for the sampling. You really did unimaginable work.

I'm greatly indebted to the New Zealand government through NZAID and the International Student Support Office (ISSO) for funding my studies. Thanks to the team leaders Ms Hooker, S. and Flynn, S. on behalf of all the team members for making my study successful in terms of perfect living and studying environment.

I extend my thanks to the government of United Republic of Tanzania through the officer-in-charge - Veterinary Investigation Centre – Northern Zone, Arusha: Dr. Swai, E., for allowing me pursuing my studies.

Thanks to my parents Andrew Ndemino and Theresa Mahoo Mshanga as well as the rest of my family for your love, care, patience and encouragement during my studies. I was away from you but I could feel your presence at all the times. Mom, you didn't live to see this work produced, but I salute and respect your contribution towards these achievements.

Thanks to chaplaincy Ms Kathleen and father(s) Brian, Marcus & Sherry for your spiritual care. Thanks Mr. McGrath, T. for enabled me to travel back home in my country for the burial ceremony of my mom. Attending the funeral and see my mom resting in peace reduced my stress and pressure eventually enabled me to settle and finish this work.

I greatly express my gratitude to Massey University particularly to IVABS for the research funds and academic guidance - Ms Allain Scott, you deserve many thanks on behalf of all the staff.

Thanks also to Mr Troy Rhodes of PGG-Wrightsons Ltd for organising the bulk of the funding for this project from Meat and Wool NZ Ltd.

To my fiancée Eva Nangela, thank you for postponed our wedding plans to allow for my studies as well as for your unlimited support and unconditional love. Thanks for your understanding while I was sometimes not reachable in the telephone, particularly at the end of this work.

Thank you all the people I met in New Zealand in your various capacitances for making my living easy and pleasing. I believe I'm not such perfect in living with you, but through your patience, guidance and care it was possible. Well wishes to you all and see you again.

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LIST OF ACRONYMS AND SYMBOLS

α	Alpha
AR	Anthelmintic resistance
β	Beta
BZ	Benzimidazole
$^{\circ}\text{C}$	Temperature in degrees centigrade
CET	Controlled efficacy test
Cm^3	Cubic metres
Co.	Company
DMSO	Dimethyl sulfoxide
DR	DrenchRite
EBSS	Earle's balanced salt solution
EC	<i>E. Coli</i> bacteria
ECYE	<i>E. coli</i> yeast extract
EHA	Egg hatch assay
FEC	Faecal egg count
FECRT	Faecal egg count reduction test
FITC	Fluorescein isothiocyanate
g	Weight in grams
GABA	Gamma-aminobutyric acid
GIN	Gastrointestinal nematodes
GIT	Gastrointestinal tract
IC_{50}	Dose response curve ₅₀
ISSO	International Student Support Office
IV	Ivermectin
IVABS	Institute of Veterinary, Animal and Biological Sciences
kg	Weight in kilograms
LDA	Larval development assay
LD_{50}	Lethal dose ₅₀

LFIA	Larval feeding inhibition assay
LV	Levamisole
ML	Macrocyclic lactones
mg	Weight in milligrams
ml	Volume in millilitres
µg	Weight in micrograms
µl	Volume in microlitres
µm	Diameter in micrometres
ng	Nanograms
No. / No.s	Number / Numbers
NZ	New Zealand
NZAID	New Zealand Aid for International Development
PBS	Phosphate buffer saline
PCR	Polymerised chain reaction
PI	Post infection
SOP	Standard operational procedure
TBZ	Thiabendazole
UK	United Kingdom
USA	United States of America
WAAVP	World Association for Advanced Veterinary Parasitology
Xg	Centrifugation revolution in gravity
γ	Gamma
YE	Yeast extract

