Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

SOME ASPECTS OF THE POPULATION DYNAMICS OF COOPERIA ONCOPHORA

A thesis presented in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in Veterinary Science

At Massey University, Palmerston North, New Zealand

Christian Wilhelm Sauermann 2014

Abstract

To develop better control strategies for *Cooperia oncophora* a detailed understanding of the population dynamics is desirable. To achieve this, aspects of the life cycle have been studied in a series of experiments, including the free-living and the parasitic phases.

To investigate the development from egg to third stage larvae, faeces containing *C. oncophora* eggs gathered from different donor animals were incubated at different constant and variable temperatures as well as under natural conditions in the field. To examine the survival of *C. oncophora* third stage larvae they were kept under similar conditions as for the development experiments. Based on the results at constant temperatures, parameters were calculated for a model to simulate the effect of temperature during the free-living phase of this nematode. A further experiment was conducted over an 11 month period to investigate the effect of host age and previous exposure on the establishment rate of third stage larvae in 3 groups of young calves. Two groups received a high or low dose of trickle-infection and the third remained as an uninfected control group.

At lower temperatures the development rate and success from egg-third stage larvae were both low but increased with higher temperatures. At 8°C 50% development was reached in 56 days with a success rate of 5.5% compared to 5 days and 26.4% respectively at 32°C. The highest development success rate of 37.4% was observed at 28°C. For larval survival, the median survival was 512.2 days at 8°C and decreased to 6.4 days at 37°C. Both development and survival were significantly (p<0.05) influenced by the host animal from which the faeces were sourced. Utilising these parameters in a model provides a useful tool to further understand the effect of temperature on the free-living stages.

The establishment rate of *C. oncophora* in the trickle-infected groups declined rapidly compared to the control group but was not significantly different (p>0.05) to the control group if the existing worm burden was removed before challenge. A decline in

establishment rate over the 11 month period of the experiment in the control animals was due to the age of the larvae.

Acknowledgements

This PhD project would not have been possible without the help and contribution of a great number of people and organisations. I would like to thank Massey University and AgResearch for giving me the opportunity of this research and the Foundation for Research, Science and Technology, New Zealand for funding me.

First I would like to thank my three supervisors for their guidance and motivation. The support they provided in planning and conducting of the experiments was invaluable. Bill Pomroy, as my chief supervisor, had the questionable pleasure of helping me with the editing of this thesis and was always a great source of knowledge making the work both instructive and interesting at the same time. Dave Leathwick, with his direct approach to problems and with his critical scientific thinking made designing experiments an entertaining exercise. I am particularly grateful for access to the model developed by Dave which I was able to use with minor modifications for simulating development and survival of *C. oncophora*. Ian Scott helped in the field many times when manpower was short, with reviewing documents and always found some time when I came with problems or questions.

From all people I have been working with I have to specially thank Anne Tunnicliffe, Barbara Adlington and Louise Shaw from the parasitology laboratory at Massey University. Without Anne's constant help the field work would not have been manageable. The expertise of Barbara has been an invaluable help in the laboratory and without her would have been impossible. Barbara and Anne were both involved with undertaking a number of faecal egg counts, larval cultures, intestinal worm counts and abomasal digests throughout the course of this study. I would also like to express my personal thanks for not only welcoming me as an international student but also my family. The help Louise provided with the processing of animal samples was greatly appreciated. All three of them made working in the parasitology lab a pleasant experience.

Tania Waghorn, Chris Miller, Lawrie McMurtry and Dean Reynecke at AgResearch provided help with the experiments. Tania provided motivating cheer-ups and help

with the design and conducting of the field experiments. I have to thank Chris for organising the husbandry for some cattle at Flock House and the baleage for the large animal trial. Lawrie provided millions of ivermectin-susceptible infective larvae. Dean Reynecke provided useful help with the cattle experiment. I am also grateful for AgResearch for facilitating access to the susceptible isolate used in Chapters 5 and 6.

John Koolaard provided useful statistical help with the design of the animal experiment on establishment. Siva Ganesh helped with the statistical analysis of the larval experiments.

Most cattle during this work have been kept on the Massey Farm, Tuapaka and I have to thank Phil Brooks and Jonathan Brophy for everyday husbandry. I also have to thank Mike Hogan for his help in the PM room at Massey University.

Of my fellow students I have to thank Ben Bauer and Sharifah Salmah Syed-Hussain who have been a great source of help and motivation. Furthermore various unnamed students from Massey University helped with laboratory and field work.

I thank Steve Pilkington, although never directly involved in this thesis, for pointing out a certain possible PhD position a couple of years ago.

Last but not least I would like to thank my family. First of all my parents and my sister for nurturing my curiosity of how the world works and always supporting my on-going endeavour into natural science. Above all, I would like to thank my wife Patricia and my kids Malte and Johanna for their tolerance during the last years. To greatly appreciate their acceptance of my everlasting devotion to science would not be adequate of how I feel. To them all my love and gratitude.

Our responsibility is to do what we can, learn what we can, improve the solutions, and pass them on.

Richard P. Feynman

Table of contents

ABSTRACT	III
ACKNOWLEDGEMENTS	V
TABLE OF CONTENTS	VII
LIST OF FIGURES	ΧI
LIST OF TABLES	XIII
LIST OF PLATES	xv
PREAMBLE	1
CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW	3
1.1 Introduction	3
1.2 Parasitic nematodes in cattle	4
1.3 The nematode Cooperia oncophora	5
1.3.1 Polymorphism in Cooperia oncophora	6
1.3.2 Life cycle overview	7
1.4 The free living phase	8
1.4.1 Development of egg to [L3]	9
1.4.1.1 The effect of temperature on eggs	9
1.4.1.2 The effect of egg size on hatching	11
1.4.1.3 The effect of temperature on egg to [L3] development	12
1.4.1.4 The effect of the host on development	13
1.4.1.5 Other effects on development	13
1.4.2 The third stage larvae – [L3]	14
1.4.2.1 Dispersal of the [L3]	15
1.4.2.2 Survival of the [L3]	17
1.4.3 Seasonal patterns	18
1.5 The parasitic phase	19
1.5.1 Establishment of the nematode in the host	19
1.5.2 The adult nematode in the host	21
1.5.3 Development of the host immune response	23
1.6 Anthelmintics	25
1.6.1 The different classes and active ingredients	25
1.6.2 Pharmacokinetics	27
1.6.3 Resistance to anthelmintics	28
1.6.4 Reason for resistance and strategies to delay it	30
1.7 Modelling	31
1.8 Conclusion	33

	PTER 2 THE EFFECT OF TEMPERATURE ON DEVELOPMENT OF THE FRE	E- 36
2.1	Abstract	36
2.2	Introduction	37
2.3	Materials and Methods	38
2	3.1 Experiments	38
	2.3.1.1 Experiment 1 - Development at constant temperatures	39
	2.3.1.2 Experiment 2 - Development at variable temperatures	39
	2.3.1.3 Experiment 3 - Development under natural conditions	40
2	3.2 Parasitological techniques	41
	2.3.2.1 Culturing faecal samples	41
	2.3.2.2 Counting techniques	41
	2.3.2.3 Recovering larvae from samples	41
2	3.3 Statistical methods	42
2.4	Results	43
2	4.1 Experiment 1 - Development under constant temperatures	43
2	4.2 Experiment 2 - Development under variable temperatures	45
2	4.3 Experiment 3 - Development under natural conditions	47
2.5	Discussion	48
2.6	Conclusion	52
2.7	Acknowledgements	52
ON	PTER 3 THE EFFECT OF TEMPERATURE ON THE SURVIVAL OF COOPERS OPHORA THIRD STAGE LARVAE Abstract	<i>1A</i> 56 56
3.2	Introduction	56
3.3	Materials and Methods	57
3	3.1 Experiment 1 - Survival at constant temperatures	57
3	3.2 Experiment 2 - Survival at variable temperatures	58
3	3.3 Experiment 3 - Survival under natural conditions	59
3	3.4 Statistical methods	60
3.4	Results	60
3	4.1 Experiment 1 - Survival at constant temperatures	60
3	4.2 Experiment 2 - Survival at variable temperatures	64
3	4.3 Experiment 3 - Survival under natural conditions	64
3.5	Discussion	66
3.6	Conclusion	69
3.7	Acknowledgements	69
	PTER 4 MODELLING THE DEVELOPMENT AND AGEING OF COOPERIA	7.4
_	OPHORA THIRD STAGE LARVAE Abstract	74 74
		74 75
	Introduction Materials and Methods	75 75
		75
2	3.1 Egg to L3 Development Model (DM) 4.3.1.1 Development rate (d _t)	75 76
	4.3.1.2 Survival rate during development (s _t)	76 76

4.3.2	L3 Ageing Model (AM, ageing rate a _t)	77
4.3.3	Evaluating the model with experimental results	78
4.3.4	Application of the model	79
4.3	4.1 Monthly L3 development and ageing over a 10 year period	79
4.3	4.2 L3 development and lifespan with variable temperatures	79
4.3.5	Statistical Methods	79
4.4 Res	ults	80
4.4.1	Model parameters	80
4.4.2	Evaluating the DM and AM	80
4.4.3	Monthly L3 development and ageing over a 10 year period	83
4.4.4	Egg to L3 development and L3 lifespan with variable temperatures	83
4.5 Dis	cussion	85
4.6 Cor		90
4.7 Ack	nowledgements	90
CHAPTE	R 5 EFFICACY OF IVERMECTIN ON TWO ISOLATES OF COOPERIA	
ONCOPE	HORA	93
5.1 Abs		93
	oduction	93
	terials and Methods	94
	Cooperia oncophora isolates	94
	Experimental animals	95
	Experimental Design	95
	Statistical analysis	96
5.4 Res		96
5.5 Dis		98
5.6 ACK	nowledgements	100
CHAPTE	R 6 ESTABLISHMENT OF COOPERIA ONCOPHORA IN CALVES	102
6.1 Abs	stract	102
	oduction	103
6.3 Ma	terials and Methods	104
6.3.1	Experimental overview	104
	Experimental animals and Husbandry	106
	Infective larvae	107
6.3.4		108
6.3.5		109
	Worm length and fecundity	109
	Statistical analysis	110
6.4 Res		110
	Baleage and shaving samples	110
	Establishment Sov ratio	111
	Sex ratio	112
6.4.5	Worm length Fecundity	113 113
6.4.6	•	113
	Egg to [L3] development rate	114
J. T. /	LAA CO LEGI GEVELOPITIETIE IULE	

Table of contents

6.4.8 Weight gain	115
6.5 Discussion	115
6.5.1 Establishment	115
6.5.2 Length, fecundity and proportion of female worms	119
6.5.3 Egg viability	120
6.5.4 Weight gain	120
6.6 Conclusion	121
6.7 Acknowledgements	121
CHAPTER 7 GENERAL DISCUSSION	125
CHAPTER 8 REFERENCES	133
CHAPTER 9 APPENDICES	151
APPENDIX 1 STANDARD OPERATING PROCEDURES	153
APPENDIX 2 SUPPLEMENTARY INFORMATION FOR CHAPTER 2	195
APPENDIX 3 SUPPLEMENTARY INFORMATION FOR CHAPTER 3	223
APPENDIX 4 SUPPLEMENTARY INFORMATION FOR CHAPTER 4	235
APPENDIX 5 SUPPLEMENTARY INFORMATION FOR CHAPTER 5	243
APPENDIX 6 SUPPLEMENTARY INFORMATION FOR CHAPTER 6	247

List of figures

Figure 1.1 - The life cycle of <i>Cooperia oncophora</i>	7
Figure 2.1 - Cooperia oncophora third stage larvae development at different constar temperatures	nt 44
Figure 2.2 - Cooperia oncophora egg to third stage larvae development at 16°C in faecal cultures prepared from 3 different hosts	44
Figure 2.3 - Regression lines for the development of <i>Cooperia oncophora</i> third stage larvae	46
Figure 2.4 - Development of <i>Cooperia oncophora</i> third stage larvae under variable temperatures	46
Figure 2.5 - Development of <i>Cooperia oncophora</i> third stage larvae under natural conditions in the field	47
Figure 3.1 - Survival of <i>Cooperia oncophora</i> third stage larvae at different constant temperatures	61
Figure 3.2 - Regression lines for 95, 50 and 5% survival of <i>Cooperia oncophora</i> third stage larvae at different constant temperatures	62
Figure 3.3 – Survival of <i>Cooperia oncophora</i> third stage larvae from different host animals kept at 24°C	63
Figure 3.4 - Comparison between survival of <i>Cooperia oncophora</i> third stage larvae a calculated survival based on exponential equations	and 64
Figure 3.5 - Proportion of recovered <i>Cooperia oncophora</i> third stage larvae from pasture plots following artificial contamination	65
Figure 4.1 a, b and c - Estimated hourly development (a) and survival (b) rates and L ageing rate (c) of <i>Cooperia oncophora</i> compared to experimental results	3 81
Figure 4.2 a, b and c - Comparison of model estimates with experimental data	82
Figure 4.3 a, b and c - Development model estimates using temperature data from January 2002 to December 2011	84
Figure 4.4 a, b and c - Model estimates for the development, lifespan and a combination for <i>Cooperia oncophora</i> L3 with oscillating temperatures	86
Figure 6.1 - a) Cooperia oncophora establishment and worm burden in young calves and b) mean faecal egg counts	112

List of tables

Table 1.1 – Common parasitic nematodes in cattle	5
Table 1.2 – <i>Cooperia</i> classification	6
Table 1.3 – Average size of development stages of <i>C. oncophora</i>	8
Table 1.4 – Development time for different stages of <i>C. oncophora</i>	20
Table 1.5 – Overview of anthelmintics	25
Table 1.6 – Ivermectin efficiency against internal and external cattle parasites	27
Table 2.1 - Individual host and mean success rates for Cooperia oncophora development at different temperatures	45
Table 3.1 - Regression equations for 95, 50 and 5% survival of <i>C. oncophora</i> third stallarvae at different constant temperatures	age 62
Table 3.2 - The effect of the host animal on survival of <i>C. oncophora</i> L3 kept at constant temperatures	63
Table 3.3 – C. oncophora third stage larvae recovered from pasture plots	65
Table 4.1 - Parameters for L3 development (DM) and ageing (AM) models	80
Table 5.1 - Worm count data for determining the efficacy of ivermectin of two <i>C. oncophora</i> isolates	97
Table 6.1 - Infection schedule for <i>C. oncophora</i> establishment experiment	105
Table 6.2 - The proportion, length and fecundity of female C. oncophora recovered from calves	113

List of plates

Plate 1 – Adult gravid <i>C. oncophora</i> female with eggs in utero	54
Plate 2 – Fully developed <i>C. oncophora</i> third stage larvae	72
Plate 3 - Adult gravid <i>Cooperia oncophora</i> female showing the vulva and part of the	
ovijector with eggs	124

List of Abbreviations

Abbreviation Description
°C Degree Celsius
μg Microgram
μm Micrometre

AM Ageing model for *C. oncophora* third stage larvae

AR Anthelmintic resistance

cm Centimetre

DM Development model for *C. oncophora* egg to third larval stage

DT50 Time to reach 50% development

epg Eggs per gram
FEC Faecal egg count

FMC Faecal moisture content

g Gram
h Hours
IVM Ivermectin

L1-L4 Larvae of stage 1-4

L3, [L3] Ex- and ensheathed third stage larvae

LT50 Median survival time

m Metre
mg Milligram
min Minutes
ml Millilitre

ML Macrocyclic lactone

nm Nano metre

R² Coefficient of determination

RES Ivermectin-resistant *C. oncophora* isolate

SEM Standard error of the mean

sg Specific gravity

SUS Ivermectin-susceptible *C. oncophora* isolate

UV Ultraviolet light

WAAVP World Association for the Advancement of Veterinary Parasitology

Preamble

The results of a national survey of beef cattle farms on the North Island of New Zealand in 2004/2005 indicated that *Cooperia* spp. was the most abundant species of parasitic nematode and was already showing a high prevalence of resistance to ivermectin and benzimidazole anthelmintics. At that time there was no resistance to levamisole but this was considered an imminent risk. For the development of future control strategies a more detailed knowledge of the dynamics of this species was necessary. In 2008 the research involved with this thesis was commenced as a cooperation of Massey University and AgResearch Grasslands in Palmerston North, New Zealand. The funding was provided by the Foundation for Research Science and Technology under contract C10X0714.

From the beginning of October 2008 a group of six young calves were kept on the Massey University Farm Tuapaka on a weekly rotation of oral ivermectin treatment and re-infection with larvae to maintain a constant source of ivermectin-resistant Cooperia oncophora. The first experiment commenced in October 2008 and investigated the survival of third stage larvae kept under constant temperatures as this was expected to continue for a significant time. The development of egg to third stage larvae at constant temperatures was commenced early the following year. The number of constant temperatures in this experiment required this experiment to be split in two parts as the time needed to analyse the samples was a limiting factor. The survival of third stage larvae in the field was investigated in June 2009 (winter) and larval contaminations continued once a month for all seasons to March 2010. The first animal experiment investigating the efficacy of ivermectin for two C. oncophora isolates took place in the first half of 2010 and was repeated with another six animals in the second half of 2011, whereas the establishment of C. oncophora in calves was examined from September 2010 to September 2011. The last experiments on development of eggs to third stage larvae and the survival of the larvae at variable temperatures in the laboratory were conducted from May 2011 onwards.

All experiments involving the use of cattle have been approved by the Massey Animal Ethics Committee or the AgResearch Animal Ethics Committee.

Several chapters of this thesis (Chapter 2, 3, 4 and 6) describing different aspects of the life-cycle of *C. oncophora* are, or will be, submitted as publications in peer reviewed journals. All Chapters 2-6 are therefore included in a publication style format. However, the formatting as well as the style of the charts and figures have been partly changed for the purpose of being integrated into one document with section numbers etc. aligned to the particular chapter number in the thesis. These changes include also cross-references between sections and to additional information in the appendices. All references have been integrated into a single reference section.