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Studies on the epidemiology of
Nematodirus spathiger and *Nematodirus filicollis*
in New Zealand

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Abstract

This thesis studies aspects of the epidemiology of *Nematodirus spathiger* and *Nematodirus filicollis*, both are common and potentially pathogenic parasites of lambs in New Zealand. Three studies were undertaken; the first a presence/absence survey to determine the distribution of the two species on farms, the second examined the prevalence of benzimidazole (BZ) resistance in the two species and the third experiment investigated the requirements for chilling for *N. filicollis* eggs to hatch.

The first two studies utilised faecal samples from routine faecal egg counts and/or routine faecal egg count reduction testing on farms. To identify the *Nematodirus* species involved, eggs from these samples were chilled and then incubated to facilitate hatching of *N. filicollis*, before larvae were recovered. The identity of the larvae was determined using PCR of the ITS-2 region of rDNA. The third experiment used a bulk collection of *N. filicollis* from naturally infected lambs. Extracted eggs were incubated at temperatures between 2.7°C - 9.9°C, for up to 224 days. The proportion of eggs hatching was assessed against chill units. Chill units (degree-day) were calculated by subtracting the culture temperature from a constant threshold of 11°C and multiplying by the number of days for which the sample was cultured.

In Study 1, *N. spathiger* was present on all farms tested, while *N. filicollis* was found on 76% of farms. Both species were distributed throughout New Zealand, with no regional differences. In Study 2, the BZ-resistance study, efficacies below 95% were recorded for *N. spathiger* and *N. filicollis* on 95% (20/21) and 40% (4/10) respectively of farms tested. In Study 3, the chilling experiment, the overall hatching of *N. filicollis* eggs was low, but increased with chill accumulation to plateau at about 11%, with 800-1000 chill units required for maximum hatching.

In conclusion the two species of *Nematodirus* were commonly found on most New Zealand farms. The prevalence of BZ-resistance in *N. filicollis* was lower than that in *N. spathiger*. *N. filicollis* required a considerable period of chilling to enable hatching to occur and this will influence their epidemiology.

Preface

In 2009-2010 two new anthelmintic families (monepantel and derquantel) were released to the market in New Zealand, which reduced the need for research into finding solutions to the anthelmintic resistance problem. It also created an opportunity to focus on minimising the impact of parasites in sheep through improved management strategies. In particular targeting specific nematodes species and understanding their epidemiology on pasture. This is poorly understood in some species. For this study the genus *Nematodirus* was targeted because it is pathogenic, it is often reported as resistant to drenches and it is found nation-wide. Further, the ecology of the genus is quite different to the other strongyle species and it has been poorly studied in New Zealand. Consequently this project was initiated with an aim of discovering the factors influencing the development and survival of the pre-parasitic stages of *Nematodirus spathiger* and *N. filicollis* and was undertaken as part of a Foundation for Research Science and Technology grant, under contract C10X0714.

The study commenced in November 2010, with a study on the effect of three temperature regimes using mixed *Nematodirus* species from different farms in tissue culture well plates. However this was unsuccessful, as many eggs did not hatch, therefore this experiment is not presented. The project commenced in late spring - early summer, when *Nematodirus* eggs are present in faeces, before a literature review could be undertaken; this resulted in not fully understanding the development and hatching requirements of *Nematodirus* and the failure of the first experiment.

At the same time faecal samples were collected and incubated from farms throughout the country for the distribution survey. Drench tests were also being sent to the laboratory for routine faecal egg count reduction testing (FECRT), which became part of the benzimidazole resistance study. As eggs had not hatched in the first experiment it was decided to halve the cultures from both these experiments. This allowed one half to be chilled and the other half processed.

Finally with the comprehension that *N. filicollis* eggs required significant chilling before they would hatch, an experiment was planned to determine what these requirements were. In late January 2012 an almost pure strain of *N. filicollis* was found on a local farm. After isolation from the field and a period of incubation to allow the eggs to

develop, the chilling experiment began in May 2012 with the eggs being placed at different temperatures.

The chapters relating to the experimental studies are presented as papers for journal publication. Chapter 3 has been published in the New Zealand Veterinary Journal (Oliver *et al.* 2014). My contribution for this published paper was 85%. Chapter 4 has been prepared for submission to the same journal; my contribution for this paper was 80%. Chapter 5 has been submitted to Veterinary Parasitology, my contribution for this paper was 75%. As a result there is some repetition of methods; also reformatting and modifications to these chapters by way of annotations and the re-numbering of sections and illustrations have been made, to reflect thesis style. Some raw data is also presented for Chapter 3, 4 and 5 in the appendices. The references for each chapter have been collated into the bibliography.

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Abbreviations

ABBREVIATION	DESCRIPTION
°C	Degree Celsius
µm	Micrometre
µL	Microlitre
ABZ	Albendazole
BZ	Benzimidazole
cm	Centimetre
CU	Chill units
DNA	Deoxyribonucleic acid
epg	Eggs per gram
FEC	Faecal egg count
FECR	Faecal egg count reduction
FECRT	Faecal egg count reduction test
g	Gram
HPLC	High performance liquid chromatography
ITS	Internal transcribed region
lb	Pound
kg	Kilogram
L2	Second stage larva
L3	Third stage larva
ML	Macrocyclic lactone
mL	Millilitre
mm	Millimetre
%	Percent
PCR	Polymerase chain reaction
pmol	Picomole
rDNA	Ribosomal Deoxyribonucleic acid
sg	Specific gravity
SOP	Standard operating procedure
SSU	Small subunit