

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.

2

A SURVEY OF ANTHELMINTIC RESISTANCE AND PARASITE
MANAGEMENT PRACTICES ON SHEEP FARMS IN
NEW ZEALAND

A DISSERTATION PRESENTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF MASTER OF
VETERINARY STUDIES (VETERINARY PARASITOLOGY) AT
MASSEY UNIVERSITY

SHYAM SUNDAR SHARMA

JULY, 2004

ABSTRACT

The occurrence of anthelmintic resistance in sheep nematodes in New Zealand was conducted by using DrenchRite and Inhouse larval development assays (LDA) on 25 randomly selected farms. Samples from 6 farms were put onto both DrenchRite and Inhouse LDA plates and were available for comparison. Both showed a similar LC_{50} and LC_{50} well for benzimidazoles but not for levamisole. Including results from both assay systems it is concluded that anthelmintic resistance in *Trichostrongylus* (either suspected or high level) was demonstrated in 60% of the farms (9/15) to benzimidazole (BZ), resistance to levamisole (LEV) in 66% of farms (10/15), combination drench (BZ+LEV) on 43% of farms (3/7) and avermectin on 1 of 8 farms. For those farms where *Trichostrongylus* was the predominant genus there was resistance to at least one anthelmintic on all tested farms (n=12). A survey of parasite control procedures over 2002/2003 was conducted on 38 farms. The principle findings were: about 58% farmers (n=37) performed quarantine drenching of brought-in sheep for which a majority of farmers (52%) used macrocyclic lactones alone or in combination with other anthelmintics; about 78% (n=37) of farmers followed a 5-6 drench programme to lambs/hoggets; the annual frequency of anthelmintic treatments (n=38) were 6.1 drenches to lambs/hoggets, 1.4 to two-tooths and 1.8 to mature ewes; about 71% farmers used macrocyclic lactones for lambs/hoggets on at least one occasion either alone or in combination with other anthelmintics; a majority (61%) of farmers followed a planned annual drench family rotation; about 76.5% (29/38) farmers had never carried out a test for drench resistance and about 57% (4/7) of those farmers who had tested did identify resistance on their farms to either the benzimidazole or combination (benzimidazole + levamisole) drench. Worms were considered "very important" by 55% farmers (n=38) for the cause of economic losses in their farm, whereas 42% farmer considered it "important". Drench resistance was considered as a "serious problem" today for the industry by 47% farmers (n=38), whereas, 34% farmers believe it as a problem but "not serious one today" for the industry.

ACKNOWLEDGEMENTS

I am grateful to my to my chief supervisor, Dr. W. E. Pomroy for his valuable guidance, encouragement, patience, helping attitude at all times and friendly behaviour during my study and research works. I would like to thank to my co-supervisors Dr. Maurice Alley and Dr. Nigel Perkins for their help and guidance.

This study and research would not have been possible without the help of various organizations/agencies. I am thankful to His Majesty's Government of Nepal and Ministry of Foreign Affairs, New Zealand for NZODA, post graduate award scholarship; Cooper's Animal Health (Schering Plough) and Institute of Veterinary Animal and Biomedical Sciences (IVABS) for research funds.

I would like to thank team leader, Silvia Hooker, Sue, Flyn and all other staff of International Students Office, Massey University for their help.

I would like to thank Mrs Barbara Adlington and Mrs Anne Tunnicliffe for their technical assistance and help provided to me during my study and research at Veterinary Parasitology laboratory.

I am grateful to my parents, my wife Bandana Poudyal Sharma, daughter Shreeya, and son Pranav for their patience and support.

Finally, I wish to express my gratitude to all those persons who encouraged and helped me during my study and stay in New Zealand.

I wish great success for all round development of Massey University and New Zealand.

TABLE OF CONTENTS

page	
ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF FIGURES	vi
LIST OF PLATES	vii
LIST OF TABLES	vii
LIST OF APPENDICES	viii
Chapter one: Introduction and literature review	
1.1. Introduction	1
1.2. Incidence of anthelmintic resistance in sheep and goats	2
1.3. Anthelmintics	6
1.3.1. Broad-spectrum anthelmintics	9
1.3.1.1. Benzimidazoles	9
1.3.1.2. Mode of action and mechanism of resistance	9
1.3.1.3. Imidazothiazoles/tetrahydropyrimidines	11
1.3.1.4. Mode of action and mechanism of resistance	12
1.3.1.5. Macrocyclic lactones	12
1.3.1.6. Mode of action and mechanism of resistance	15
1.4. Definitions of anthelmintic resistance	17
1.5. Anthelmintic resistance in sheep and goats in New Zealand	19
1.6. Diagnosis of anthelmintic resistance	21
1.6.1. <i>In Vivo</i> tests	21
1.6.1.1. Faecal egg count reduction test (FECRT)	21
1.6.1.2. Controlled efficacy test	22
1.6.2. <i>In vitro</i> test	22
1.6.2.1. Egg assay test for benzimidazoles	23
1.6.2.2. Egg hatch assay test for levamisole	23
1.6.2.3. Larval development assay (LDA)	24
1.6.2.4. Adult development test	27
1.6.2.5. Larval paralysis and motility assay	27
1.6.2.6. Biochemical tests	28
1.6.2.7. Molecular and genetic techniques	28
1.7. Comparison of various techniques for LDA	29
1.8. Epidemiology of nematodes in New Zealand	31
1.9. Nematode control measures	34

Chapter Two: Survey on anthelmintic resistance on various sheep farms in New Zealand, using larval development assays

2.1. Introduction	37
2.2. Materials and methods	38
2.2.1. Sample collection and egg recovery	38
2.2.2. DrenchRite LDA	39
2.2.3. Larval culture	41
2.2.4. Inhouse LDA	41
2.2.5. Data analysis	43
2.3. Results of DrenchRite and Inhouse assays	44
2.5. Discussion	54

Chapter Three: Survey on anthelmintic resistance and parasite control practices on sheep farms in New Zealand: a questionnaire survey

3.1. Introduction	60
3.2. Material and methods	61
3.2.1. Selection of sheep farms and questionnaire design	61
3.3. Statistical analysis	62
3.4 Results	62
3.5 Discussion	76

Chapter Four: General discussion 83**References** 144

LIST OF FIGURES

Figure 1.1: The inter-relationship between the pasture contamination by untreated lambs and ewes and infective larvae from pasture.	32
Figure 1.2: Effects of monthly preventive drenches from weaning on the faecal output of lambs and pattern of larval availability on pasture.	35
Figure 2.1: Bar chart showing Inhouse LDA, LC ₅₀ wells from various farms for BZ, LEV and ivermectin aglycone.	49
Figure 2.2: Bar chart showing LC ₅₀ wells from various farms for BZ, LEV, COMB and ivermectin aglycone.	49
Figure 2.3: showing normal probability plot for comparisons of DrenchRite and Inhouse LC ₅₀ for benzimidazole and levamisole.	51
Figure 2.4: Showing a typical dose response curve relating to the proportion of L3s of <i>Trichostrongylus</i> spp. to log ₁₀ concentration (µM) of thiabendazole.	54
Figure 3.1: Showing quarantine drenching done in various Sheep farms.	64
Figure 3.2: Same drench use in sheep farms.	65
Figure 3.3: Grazing management for lambs.	66
Figure 3.4: Proportion of lambs weaned onto paddocks not grazed by lambing ewes.	66
Figure 3.5: Average time taken before lambs moved to paddocks that was grazed by ewes and lambs	67
Figure 3.6: Cattle/deer grazing between period of lamb grazing	67
Figure 3.7: Proportion of farm grazed by lambs	68

Figure 3.8: Showing intention of using same drench next year	70
Figure 3.9: Showing number of drenches to two tooth (1-2 yr age)	71
Figure 3.10: Showing number of drenches to ewes	71
Figure 3.11: Showing drench test done by farmers	74
Figure 3.12: Overall effectiveness of the drenching programme	75
Figure 3.13: Showing farmers views about drench resistance Problem	76

LIST OF PLATES

Plate 2.1: DrenchRite LDA plates.	40
Plate 2.2: Inhouse LDA plates.	43

LIST OF TABLES

Table 1.1: Anthelmintic resistance reports in sheep and goats from various continents/countries	2
Table 1.2: Anthelmintic groups	8
Table 1.3: Summary of laboratory incubation of nematode eggs	30
Table 2.1: Average FEC and percentage and percentage of different genera of trichostrongylids on larval culture from various farms	48
Table 2.2: Showing DrenchRite assay analysis results	50

Table 2.3: Showing Inhouse assay analysis results	52
Table 2.4: Showing Comparison between DrenchRite and Inhouse assay results	53
Table 3.1: Details of farms and livestock numbers	63
Table 3.2: Details of management of lambs/drenching policy	63
Table 3.3: Showing use of anthelmintics in ewes before lambing	72

LIST OF APPENDICES

Appendix 2.1. Modified McMaster technique for counting of eggs	88
Appendix 2.2 Procedure for larval culture	89
Appendix 2.3 (a) Procedure for Inhouse larval development assays.	91
(b) Procedure for DrenchRite larval development assays.	100
Appendix 2.4. Statistical analysis of dose response curve and LC ₅₀ values from various farms.	
(1) DrenchRite assays (a to l)	103
(2) Inhouse assays (a to l)	118
Appendix 3.1. Standard survey questionnaire (6 pages)	133
Appendix 3.3. Drugs used in quarantine drenching.	138
Appendix 3.4. Details of anthelmintic drugs used in lambs/hoggets in 2002/2003.	140
Appendix 3.5. details of drug used in ewes in 2002.	142