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.
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 LC50 and LC50 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 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 greatful 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. In Vivo tests 21
1.6.1.1. Faecal egg count reduction test (FECRT) 21
1.6.1.2. Controlled efficacy test 22
1.6.2. In vitro 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

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1. Introduction</td>
<td>37</td>
</tr>
<tr>
<td>2.2. Materials and methods</td>
<td>38</td>
</tr>
<tr>
<td>2.2.1. Sample collection and egg recovery</td>
<td>38</td>
</tr>
<tr>
<td>2.2.2. DrenchRite LDA</td>
<td>39</td>
</tr>
<tr>
<td>2.2.3. Larval culture</td>
<td>41</td>
</tr>
<tr>
<td>2.2.4. Inhouse LDA</td>
<td>41</td>
</tr>
<tr>
<td>2.2.5. Data analysis</td>
<td>43</td>
</tr>
<tr>
<td>2.3. Results of DrenchRite and Inhouse assays</td>
<td>44</td>
</tr>
<tr>
<td>2.5. Discussion</td>
<td>54</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1. Introduction</td>
<td>60</td>
</tr>
<tr>
<td>3.2. Material and methods</td>
<td>61</td>
</tr>
<tr>
<td>3.2.1. Selection of sheep farms and questionnaire design</td>
<td>61</td>
</tr>
<tr>
<td>3.3. Statistical analysis</td>
<td>62</td>
</tr>
<tr>
<td>3.4 Results</td>
<td>62</td>
</tr>
<tr>
<td>3.5 Discussion</td>
<td>76</td>
</tr>
</tbody>
</table>

### Chapter Four: General discussion

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Referencess</td>
<td>144</td>
</tr>
</tbody>
</table>
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, LC50 wells from various farms for BZ, LEV and ivermectin aglycone. 49

Figure 2.2: Bar chart showing LC50 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 LC50 for benzimidazole and levamisole. 51

Figure 2.4: Showing a typical dose response curve relating to the proportion of L3s of Trichostrongylus spp. to log10 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