Anthelmintic Resistance in Ontario Sheep Flocks

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Outline

- Overview of Gastro-Intestinal Nematodes (GIN) and Anthelmintic Resistance
- Research Project
  - Research Objectives
  - Materials and Methods
  - Results
  - Future Plans
- Overall Summary

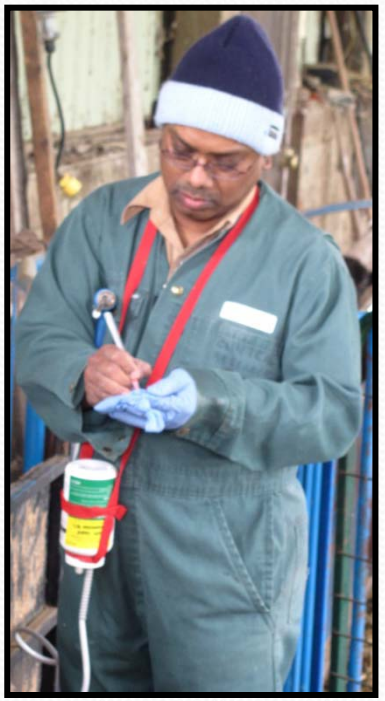
Gastro-Intestinal Nematodes (GIN)

- Parasites inhabiting the alimentary tract of small ruminants
- Responsible for severe economic losses

(Courtesy of Dr. Neil Sargison, University of Edinburgh)

Anthelmintics

- Traditionally controlled using anthelmintics and feed/pasture management
- Three major drug classes:
  - Macrocyclic lactones (eg. ivermectin)
  - Benzimidazoles (eg. fenbendazole)
  - Tetrahydropyrimidines (eg. levamisole)



Anthelmintic Resistance (AR)

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- In 2007, AR was first reported in Canada on a sheep farm in Ontario (Glauser *et al.*, 2007)

Research Objectives

1. To determine the frequency of ivermectin **Drench Failure** by performing a Drench Check

2. To determine the frequency of **Anthelmintic Resistance** using the Faecal Egg Count Reduction Test (FECRT)

3. To validate a **Larval Development Test** (LDT) and evaluate consistency of results with FECRT's

Materials and Methods

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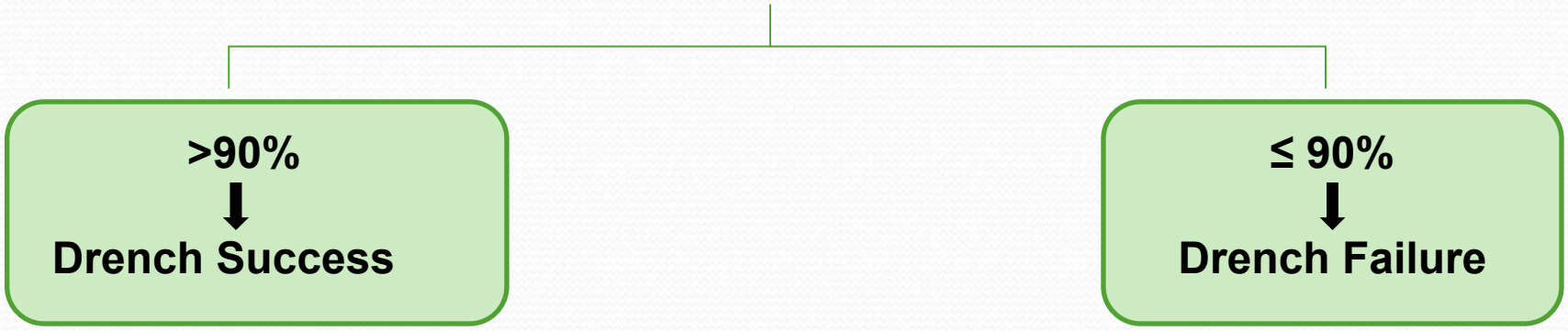
- 15 faecal samples/farm were collected from grazing animals on a monthly basis
- Faecal samples were analyzed using modified McMaster technique
- Average Faecal Egg Count (FEC) reached 200 eggs per gram (epg) threshold ⇒ Drench Check

Materials and Methods – Drench Check

- Ivermectin was sent to the producer - asked to deworm as they NORMALLY would
- 14 days post-treatment collect faecal samples – FEC
- Calculate overall reduction:



If Drench Failure - FECRT

- Animals were assigned into 2-4 treatment groups

  (15 animals/group)

  - Assigned to Untreated/Treated group
  - Identified
  - Weighed
  - Untreated/Treated with anthelmintic
  - Collected faecal samples
- 14 days later collected faecal samples

Materials and Methods - Calculations

- Overall reduction in each group was calculated using the WAAVP method:

  (World Association for the Advancement of Veterinary Parasitology, 1992)

  - Percentage Reduction = 100* (1-T2/C2)
  - 95% Confidence Intervals (C.I.)

- Resistance present if:

i) **Percentage Reduction <95% AND**

ii) **95% C.I. < 90%**

Larval Development Test (LDT)

- Recover GIN eggs from POOLED (per farm), FRESH, PRE-TREATMENT faecal samples
- Placed in culture wells with:
  - No Anthelmintics (i.e. Controls)
  - Thiabendazole (at 20mg/l and 60mg/l)
  - Levamisole (at 200mg/l and 600mg/l)
- After 7 days, count number of worms developed

Worms developing = Resistant worms

Results

- 25 farms submitted monthly faecal samples over the grazing season
- 23 farms reached the mean 200 epg threshold and the producers performed the ivermectin Drench Check
  - 2 farms dropped out
  - 5 farms had drench success
  - 16 farms had drench failure

- We performed the FECRT on 11 of these 16 farms

Results - % Reductions (100*{1-T2/C2})

Farm	Ivermectin			Fenbendazole			Levamisole		
		95% C.I.			95% C.I.			95% C.I.	
	% Reduction	Upper	Lower	% Reduction	Upper	Lower	% Reduction	Upper	Lower
1	**76**	95	-21						
2	**40**	80	-76						
3	**-93**	72	-126						
4	**29**	92	-514						
5	**-16**	33	-747						
6	**59**	78	24						
7	**-157**	7	-609						
8	**-23**	47	-185						
9	**54**	80	-5						
10	**93**	99	57						
11	**75**	88	46						

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8	**-23**	47	-185	**0**	55	-123			
9	**54**	80	-5	**34**	66	-28			
10	**93**	99	57	**74**	96	-70			
11	**75**	88	46	**-14**	43	-126			

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Results - LDT

- On Farm 10, the FECRT showed:
  - Resistance to Fenbendazole (overall reduction = 74%)
  - Susceptibility to Levamisole (overall reduction = 96%)

Treatment	L3 count					
	Haemonchus	*Teladorsagia*	*Trichostrongylus*	*Cooperia*	*Oesophagostomum*	Total
Control	25	142	12	0	0	178
Benz 20						
Benz 60						
Leva 200						
Leva 600						

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Benz 20	8	8	2	0	0	18
Benz 60	0	3	0	0	0	3
Leva 200						
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Benz 60	0	3	0	0	0	3
Leva 200	0	0	0	0	0	0
Leva 600	0	0	0	0	0	0

Future Plans

- For Summer 2011, 25 new farms have been enrolled
- Determine Risk Factors for AR
- Perform Larval Development Tests when farms reach 200epg threshold

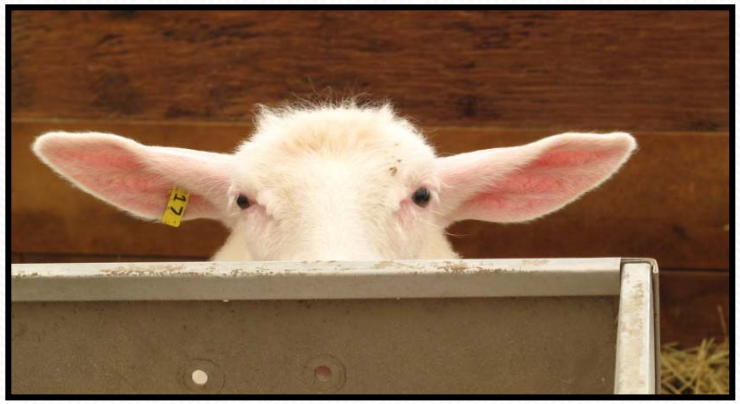
Overall Summary

- Ivermectin drench failure is much more common than we expected and most, if not all, is due to True Resistance

- Larval Development Test is confirming the results obtained with FECRT

Overall Summary

- Knowing AR status in Ontario will allow us to change management practices for parasite control
  - Encourage producers to monitor regularly
  - Provide more accessible tests for AR detection



Acknowledgements

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- Jacob Avula
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Questions?

Drench Failure or Resistance?

- There are several ways to assess resistance status:

1. **Drench Check**
   - cheaper and more practical
2. **Fecal Egg Count Reduction Test (FECRT)**
   - under controlled conditions
3. **Larval Development Test (LDT)**
   - *in vitro* assay