

Gastro-intestinal nematode resistance to anthelmintic drug classes utilizing a larval development assay and associated risk factors in Montana and Wyoming sheep

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Overview

The objective of the study was to provide baseline estimates of gastrointestinal nematode anthelmintic resistance in sheep flocks in Montana and Wyoming, utilizing a larval development assay (LDA, DrenchRite®). Additionally, fecal samples were analyzed and cultured to identify proportion of nematode species present across sheep operations. Sampling occurred on sheep operations grazing on irrigated or sub-irrigated pastures in Montana and Wyoming with a history of internal parasite challenges. Successful completion of the DrenchRite® assay requires fecal samples with a large number of *Haemonchus Contortus* (HC) larvae (>500 eggs per gram). Initially ranches were selected for the study based on word-of-mouth solicitation and a known history of internal parasite challenges. Composite fecal samples were collected June to August, 2017-2019 from 28 ranches of which 12 provided results from the DrenchRite® assay for HC. *Haemonchus Contortus* was the predominant species present across all operations sampled. Risk factors were assessed from questionnaires from participating producers where a successful DrenchRite® assay was conducted. Results indicate level of resistance is dependent on anthelmintic type. Resistance to Benzimidazole was detected on 10 of 11 operations (91%); Levamisole resistance was on 5 of 6 operations (83%); Ivermectin was on 7 of 12 operations (58%), and Moxidectin resistance was on 4 of 12 operations (33%). Results indicates parasite resistance to anthelmintics is occurring in Montana and Wyoming and is greatest concern to sheep operations grazing irrigated pastures. Results provide a small but regionally specific data set from which management recommendations can be further developed.

Results

Fecal samples were collected at 28 ranches (MT n= 20; WY n=8) which resulted in 13 successful DrenchRite® assay results to provide estimates of anthelmintic resistance. coproculture was analyzed on 26 ranches to provide an estimate of gastrointestinal nematode species present across ranches. Survey/questionnaire data was provided from a total of 16 producers yet only 12 were used to identify risk factors associated with DrenchRite® data.

Gastrointestinal nematode species composition in Montana and Wyoming sheep operations (n =26) from coproculture analysis is displayed in Table 1. The predominant species present in 74% (20 of 26) of operations sampled was the *Haemonchus Contortus* spp. which is consistent with estimates in the Eastern U.S. and Canada. Less prevalent, yet relevant species included *Teladorsagia*, *Trichostrongylus*, and *Oesphagostomum*. *Cooperia* species consistently represented a minor proportion of parasites present across operations sampled.

Table 1. Fecal egg counts (FEC), and gastrointestinal nematode species composition in Montana and Wyoming sheep operations (n =26) from coproculture analysis.

Species	Mean	Min	Max
Fecal Egg Count, eggs per gram	2,204	50	11,050
<i>Haemonchus Contortus</i> , %	69.5	2	100
<i>Trichostrongylus</i> , %	16	1	98
<i>Teladorsagia</i> , %	23.7	3	48
<i>Oesphagostomum</i> , %	22	2	54
<i>Cooperia</i> , %	6.4	1	33

Summary of resistance status for operations tested for *Haemonchus Contortus* (HC) based on results of the DrenchRite® Larval Development Assay are included in **Table 2**. Results indicate widespread resistance to Benzimidazole on operations sampled (10 of 11 operations; 91%), it's noteworthy the only operation where HC are susceptible had significantly lower FEC (200 epg). Interpretation of Levamisole resistance data is difficult due to limited experimental units (6 operations), notwithstanding (5 of 6 operations; 83%) were resistant. Interestingly all Levamisole resistant operations had not utilized Levamisole-type de-wormers in the previous 5 years. Resistance to Ivermectin was observed (7 of 12 operations; 58%), of those operations showing resistance only 57% had used Ivermectin-type de-wormers in the previous 5 years. The lowest levels of resistance were observed with Moxidectin (4 of 12 operations; 33%), of those operations showing resistance (3 of 4; 75%) had used Moxidectin-type de-wormers in the previous 5 years.

Table 2. Summary of resistance status for operations tested for *Haemonchus Contortus* based on results of the DrenchRite® Larval Development Assay¹.

12 Operations, (%) ²	Benzimidazole	Levamisole ³	Ivermectin	Moxidectin
Susceptible	1 (9%)	—	4 (33.3%)	7 (58.3%)
Suspected Resistant	—	1 (17%)	1 (8.3%)	1 (8.3%)
Resistant	10 (91%)	5 (83%)	7 (58.3%)	4 (33.3%)

¹Only represents results of *Haemonchus Contortus* DrenchRite® results. Results for *Trichostrongylus* were limited to 3 operations due to the minor proportion this species comprised across ranches. All 3 operations showed susceptibility to Benzimidazole, susceptibility to Ivermectin on 1 of 3 ranches, 2 ranches provided valid results for Levamisole with 1 being susceptible and 1 resistant.

²Susceptible = $\geq 95\%$ efficacy or no resistance to anthelmintic, Suspected Resistance = resistance to anthelmintics suspected but retesting is recommended, Resistance = $< 80\%$ efficacy or resistance to anthelmintic

³Results for *Haemonchus Contortus* for Levamisole only represent 6 operations due to assay deterioration and unreliable results. For *Trichostrongylus*, only 2 ranches provide valid results.

Table 3. Summary of questionnaire responses for operations tested for *Haemonchus Contortus* (n= 12) based on results of the DrenchRite® Larval Development Assay¹

Question	Mean	Min	Max
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Number of ewes lambing	187	32	620
Lambing, %	158	110	195
Weaning, %	148	105	180
Lamb Mortality, %	12	2	23

Question	No. of Respondents (n=12)	Percentage, %
How is your pasture irrigated?		
Sub-Irrigated	1	8
Flood	4	33
Sprinkler	5	42
Flood and Sprinkler	2	17
Grazing system?		
Continuous	6	50
Rotational	5	42
Continuous and Rotational	1	8
Estimated death loss due to internal parasites?		
0 to 5%	10	83
5 to 10%	2	17
10 to 15%	0	
Estimated % of flock performing poorly due to internal parasites?		
0 to 10%	5	42
10 to 20%	5	42
20 to 30%	1	8
30 to 40%	1	8
*Greatest internal parasite challenge?		
<i>Haemonchus Contortus</i>	6	55
Coccidia	2	18
<i>Haemonchus Contortus</i> and <i>Coccidia</i>	3	27
Is FAMACHA scoring utilized on operation?		
No	8	67
Yes	4	33
Utilize phenotypic (visual or FAMACHA) selection for parasite resistance?		
No	7	58
Yes	5	42

Table 3. Cont.

Question	No. of Respondents (n=12)	Percentage, %
Valbazen® used in previous 5 years?		
No	2	16
Yes	10	84
Safeguard® used in previous 5 years?		
No	10	84
Yes	2	16
Ivermectin® used in previous 5 years?		
No	5	42
Yes	7	58
Cyductin® used in previous 5 years?		
No	7	58
Yes	5	42
Prohibit® used in previous 5 years?		
No	10	84
Yes	2	16
Use de-wormer until ineffective?		
No	8	67
Yes	4	33
Alternate de-wormers yearly?		
No	9	75
Yes	3	25
Alternate during same season?		
No	8	67
Yes	4	33
Utilize combination de-wormers?		
No	11	92
Yes	1	8