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

W. C. Stewart<sup>\*</sup>, D. Scott<sup>‡</sup>, S. Howell<sup>†</sup>, R. Kaplan<sup>†</sup>, B. Roeder<sup>§</sup>, T. W. Murphy<sup>#3</sup>

\*University of Wyoming, Department of Animal Science, Laramie, WY ‡National Center for Appropriate Technology, Butte, MT
†University of Georgia, College of Veterinary Medicine, Kaplan Parasitology Lab, Athens, GA §Montana State University, Department of Animal and Range Sciences, Bozeman, MT
#USDA, ARS, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE 68933

## **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 Benzimadazole 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 *Oesphagostumum*. *Cooperia* species consistently represented a minor proportion of parasites present across operations sampled.

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

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

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 Benzimadazole 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 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 Assay<sup>1</sup>.

12 Operations, $(\%)^2$	Benzimidazole	Levamisole <sup>3</sup>	Ivermectin	Moxidectin
1 , ( )		Levannsole		
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%)

<sup>1</sup>Only 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.

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

<sup>3</sup>Results 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 Assay<sup>1</sup>

, , , , , , , , , , , , , , , , , , ,			
Question	Mean	Min	Max

Respondents (n=12)How is your pasture irrigated?18Sub-Irrigated18Flood433Sprinkler542Flood and Sprinkler217Grazing system?77Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal parasites?80 to 5%10835 to 10%21710 to 15%01Estimated % of flock performing poorly due to internal parasites?70 to 10%54220 to 30%18*Greatest internal parasite challenge?8Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	Number of ewes lambing	187	32	620
Lamb Mortality, %12223QuestionNo. of Respondents (n=12)QuestionPercentage Respondents (n=12)How is your pasture irrigated? Sub-Irrigated18Flood433Sprinkler542Flood and Sprinkler217Grazing system? Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal parasites? 0 to 5%10835 to 10%21710 to 15%0Estimated % of flock performing poorly due to internal parasites? 0 to 10%54220 to 30%18*Greatest internal parasite challenge? Haemonchus Contortus No655Agenetic internal parasite challenge? Haemonchus Contortus and Coccidia 327Is FAMACHA scoring utilized on operation? No867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	Lambing, %	158	110	195
Lamb Mortality, %12223QuestionNo. of Respondents (n=12)QuestionPercentage Respondents (n=12)How is your pasture irrigated? Sub-Irrigated18Flood433Sprinkler542Flood and Sprinkler217Grazing system? Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal parasites? 0 to 5%10835 to 10%21710 to 15%0Estimated % of flock performing poorly due to internal parasites? 0 to 10%54220 to 30%18*Greatest internal parasite challenge? Haemonchus Contortus No655Age433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	Weaning, %	148	105	180
Respondents (n=12)How is your pasture irrigated?Sub-Irrigated18Flood433Sprinkler542Flood and Sprinkler217Grazing system?77Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal parasites?80 to 5%10835 to 10%21710 to 15%01Estimated % of flock performing poorly due to internal parasites?80 to 10%54220 to 30%18*Greatest internal parasite challenge?8Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758		12	2	23
Respondents (n=12)How is your pasture irrigated?Sub-Irrigated18Flood433Sprinkler542Flood and Sprinkler217Grazing system?77Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal parasites?80 to 5%10835 to 10%21710 to 15%01Estimated % of flock performing poorly due to internal parasites?60 to 10%54210 to 20%54220 to 30%18*Greatest internal parasite challenge?8Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758				
(n=12)How is your pasture irrigated?Sub-Irrigated18Flood433Sprinkler542Flood and Sprinkler217Grazing system?7Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal8parasites?0100 to 5%10835 to 10%21710 to 15%0Estimated % of flock performing poorly due1to internal parasites?00 to 10%54220 to 30%1830 to 40%1*Greatest internal parasite challenge?8Haemonchus Contortus and Coccidia218 FAMACHA scoring utilized on2operation?8No8% Yes433Utilize phenotypic (visual or FAMACHA)selection for parasite resistance?7No758	Question			Percentage, %
How is your pasture irrigated?18Sub-Irrigated18Flood433Sprinkler542Flood and Sprinkler217Grazing system?7Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal parasites?00 to 5%10835 to 10%21710 to 15%010Estimated % of flock performing poorly due to internal parasites?00 to 10%54220 to 30%1830 to 40%18*Greatest internal parasite challenge?1Haemonchus Contortus and Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867No867YesVes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance?58			-	
Sub-Irrigated18Flood433Sprinkler542Flood and Sprinkler217Grazing system?77Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal parasites?00 to 5%10835 to 10%21710 to 15%0Estimated % of flock performing poorly due to internal parasites?00 to 10%54220 to 30%1830 to 40%18*Greatest internal parasite challenge?1Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867No8674Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance?7No758			(n=12)	
Flood433Sprinkler542Flood and Sprinkler217Grazing system?77Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal8parasites?0100 to 5%10835 to 10%21710 to 15%010Estimated % of flock performing poorly due10to internal parasites?00 to 10%54210 to 20%54220 to 30%1830 to 40%18*Greatest internal parasite challenge?1Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	How is your pasture irrigated?			
$\begin{array}{c cccc} Sprinkler & 5 & 42 \\ Flood and Sprinkler & 2 & 17 \\ \hline Grazing system? & & & & \\ Continuous & 6 & 50 \\ Rotational & 5 & 42 \\ Continuous and Rotational & 1 & 8 \\ \hline \\ Estimated death loss due to internal \\ parasites? & & 10 & 83 \\ 5 to 10\% & 10 & 83 \\ 5 to 10\% & 2 & 17 \\ 10 to 15\% & 0 & \\ \hline \\ Estimated \% of flock performing poorly due \\ to internal parasites? & & & \\ 0 to 10\% & 5 & 42 \\ 10 to 20\% & 5 & 42 \\ 20 to 30\% & 1 & 8 \\ \hline \\ & & & & & & \\ 30 to 40\% & 1 & 8 \\ \hline \\ & & & & & & \\ \hline \\ Haemonchus Contortus & 6 & 55 \\ Coccidia & 2 & 18 \\ Haemonchus Contortus and Coccidia & 3 & 27 \\ \hline \\ & & & & & \\ Is FAMACHA scoring utilized on \\ operation? & & & \\ No & & & & & \\ No & & & & & 7 & 58 \\ \hline \end{array}$	Sub-Irrigated		1	8
Flood and Sprinkler217Grazing system?77Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal8parasites?0100 to 5%10835 to 10%21710 to 15%0Estimated % of flock performing poorly due0to internal parasites?00 to 10%54210 to 20%54220 to 30%1830 to 40%18*Greatest internal parasite challenge?1Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867No8674Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	Flood			33
Grazing system?650Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal18parasites?010835 to 10%21710 to 15%0Estimated % of flock performing poorly due0to internal parasites?00 to 10%54220 to 30%1830 to 40%1*Greatest internal parasite challenge?Haemonchus Contortus6552218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized onoperation?No867Yes433Utilize phenotypic (visual or FAMACHA)selection for parasite resistance?No758	Sprinkler		5	42
Continuous650Rotational542Continuous and Rotational18Estimated death loss due to internal parasites?80 to 5%10835 to 10%21710 to 15%0Estimated % of flock performing poorly due to internal parasites?00 to 10%54210 to 20%54220 to 30%1830 to 40%18*Greatest internal parasite challenge?8Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867Yes4330Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	Flood and Sprinkler		2	17
Rotational542Continuous and Rotational18Estimated death loss due to internal parasites?10830 to 5%10835 to 10%21710 to 15%010Estimated % of flock performing poorly due to internal parasites?5420 to 10%54210 to 20%54220 to 30%1830 to 40%18*Greatest internal parasite challenge?8Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867Yes4330Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	Grazing system?			
Continuous and Rotational18Estimated death loss due to internal parasites?10830 to 5%10835 to 10%21710 to 15%010Estimated % of flock performing poorly due to internal parasites?5420 to 10%54210 to 20%54220 to 30%1830 to 40%18*Greatest internal parasite challenge?8Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867No8674Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	Continuous		6	50
Estimated death loss due to internal parasites?10830 to 5%10835 to 10%21710 to 15%0Estimated % of flock performing poorly due to internal parasites?5420 to 10%54210 to 20%54220 to 30%1830 to 40%18*Greatest internal parasite challenge?655Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867No8674Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	Rotational		5	42
parasites?10830 to 5%1021710 to 15%01710 to 15%016Estimated % of flock performing poorly due to internal parasites?5420 to 10%54210 to 20%54220 to 30%1830 to 40%18*Greatest internal parasite challenge?8Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	Continuous and Rotation	al	1	8
0 to 5% $10$ $83$ $5$ to 10% $2$ $17$ $10$ to 15% $0$ Estimated % of flock performing poorly due to internal parasites? $0$ $0$ to 10% $5$ $42$ $10$ to 20% $5$ $42$ $20$ to $30%$ $1$ $8$ $30$ to $40%$ $1$ $8$ *Greatest internal parasite challenge? $Haemonchus Contortus$ $6$ $Haemonchus Contortus$ and $Coccidia$ $3$ $27$ Is FAMACHA scoring utilized on operation? No $8$ $67$ Yes $4$ $33$ Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No $7$ $58$	Estimated death loss due to inte	ernal		
5 to 10%       2       17         10 to 15%       0         Estimated % of flock performing poorly due       0         to internal parasites?       42         0 to 10%       5       42         10 to 20%       5       42         20 to 30%       1       8         30 to 40%       1       8         *Greatest internal parasite challenge?       6       55 <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?       8       67         No       8       67       33         Vtilize phenotypic (visual or FAMACHA) selection for parasite resistance?       33       33         No       7       58	parasites?			
10 to 15%0Estimated % of flock performing poorly due to internal parasites?50 to 10%54210 to 20%20 to 30%130 to 40%1*Greatest internal parasite challenge?Haemonchus Contortus65522Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?No8% Selection for parasite resistance? NoNo758	0 to 5%		10	83
Estimated % of flock performing poorly due to internal parasites?5420 to 10%54210 to 20%54220 to 30%1830 to 40%18*Greatest internal parasite challenge?655Coccidia218Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?867No867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	5 to 10%		2	17
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	10 to 15%		0	
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	Estimated % of flock performin	ng poorly due		
0 to 10%         5         42           10 to 20%         5         42           20 to 30%         1         8           30 to 40%         1         8           *Greatest internal parasite challenge?         6         55           Haemonchus Contortus         6         55           Coccidia         2         18           Haemonchus Contortus and Coccidia         3         27           Is FAMACHA scoring utilized on operation?         8         67           No         8         67           Yes         4         33           Utilize phenotypic (visual or FAMACHA) selection for parasite resistance?         7         58	_			
10 to 20%54220 to 30%1830 to 40%18*Greatest internal parasite challenge?655 <i>Haemonchus Contortus</i> 655Coccidia218 <i>Haemonchus Contortus</i> and <i>Coccidia</i> 327Is FAMACHA scoring utilized on operation?867No867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance?758	-		5	42
20 to 30%1830 to 40%18*Greatest internal parasite challenge?655Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?78No867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance?758	10 to 20%			42
30 to 40%18*Greatest internal parasite challenge?655Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?766No867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758				
*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			1	8
Haemonchus Contortus655Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?766No867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758		llenge?		
Coccidia218Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?-No867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No7	_	8	6	55
Haemonchus Contortus and Coccidia327Is FAMACHA scoring utilized on operation?77No867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758				
Is FAMACHA scoring utilized on operation?8No8Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No7		nd <i>Coccidia</i>		
operation?867No867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758				, <u> </u>
No867Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No7	6			
Yes433Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758	1		8	67
Utilize phenotypic (visual or FAMACHA) selection for parasite resistance? No758				
selection for parasite resistance? No 7 58		AMACHA)	•	
No 7 58		,		
	-		7	58
$Y_{PS} = 5$ A2	Yes		5	42

Table 3. Cont.

Question	No. of	Percentage, %
	Respondents	
	(n=12)	
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 <sup>®</sup> used in previous 5 years?		
No	5	42
Yes	7	58
Cydectin® 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