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Anthelmintic Resistance in Gastrointestinal Nematodes and Associated Management Factors in Intermountain West Sheep Flocks[1-4]

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Summary

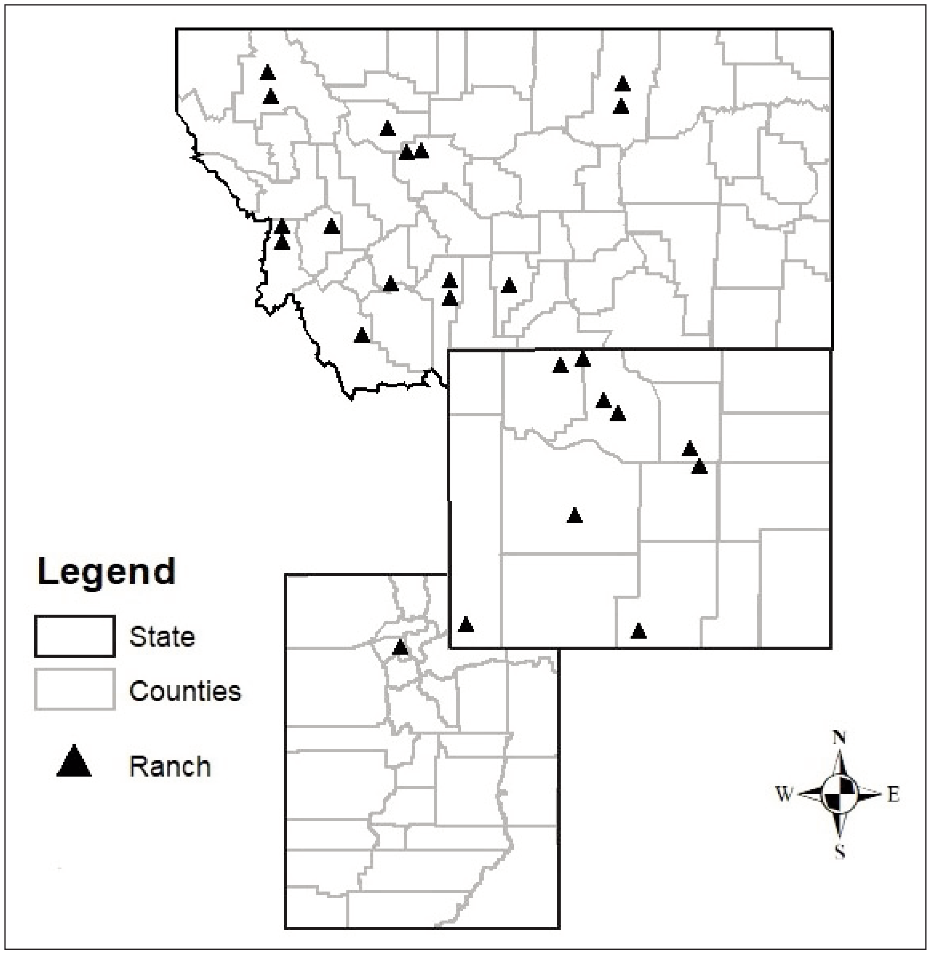
The objectives of this study were to provide baseline estimates of gastrointestinal nematode (GIN) prevalence and species composition on sheep operations grazing irrigated or sub-irrigated pastures, quantify anthelmintic resistance utilizing a commercially available larval development assay (LDA), and identify management risk factors from producer responses to survey data. Sampling occurred during the summers of 2017 to 2019 on 25 sheep operations in Montana (n = 15), Wyoming (n = 9), and Utah (n = 1). Operations were selected for the study based on word-of-mouth solicitation and limited to those with a history of GIN challenges. Fecal samples collected at each operation were composited into a single sample for coproculture and LDA analysis. Overall, *H. contortus* was the most commonly identified GIN across operations (68.5%) followed by *Trichostrongylus* (12.4%), *Oesphagostumum* (8.9%), *Teladorsagia* (8.4%), and *Cooperia* (1.8%). Twelve operations were evaluated for resistance to *H. contortus* using the LDA for benzimidazoles, ivermectin, and moxidectin. Results indicated that resistance to *H. contortus* was highly prevalent with benzimidazoles (91.7%), followed by ivermectin (50%) and moxidectin (8.3%). Grazing system and prior use of the corresponding anthelmintic class did not significantly impact *H. contortus* susceptibility to ivermectin. Questionnaire responses indicated that 56% of producers attributing production losses to GIN in 0 to 10% of their flock but only 25% utilized targeted treatment methods to guide anthelmintic administration. Results from the present study indicate that anthelmintic resistance to multiple drug classes is a concern in Intermountain West flocks that routinely utilize irrigated pastures as a forage base.

Keywords: Anthelmintic Resistance, Gastrointestinal Nematodes, Haemonchus Contortus, Irrigated Pasture, Risk Factors, Sheep

Introduction

Gastrointestinal nematodes (GIN) are significant challenges to sheep production systems worldwide (Roeber et al., 2013), and exacerbated by increasing anthelmintic drug resistance (Kaplan and Vidyashankar, 2012). A broad spectrum of economically relevant GIN species challenge small ruminant production in North America, however, published reports indicate *Haemonchus contortus* and *Trichostrongylus* spp. are of the greatest concern (Howell et al., 2008). Estimates of anthelmintic resistance in common sheep GIN have been conducted in the Eastern U.S. (Howell et al., 2008; Crook et al., 2016), Eastern Canada (Falzon et al., 2013 a & b), and South Central U.S. (Tsukahara et al., 2017), indicating regionally nuanced resistance to current anthelmintic drug classes. Regional studies estimating resistance can help guide best management practices and inform adaptive strategies to combat GIN. However, differences in climate and management systems affect the prevalence of GIN and extrapolation of results can be limited. The Intermountain West region of the U.S. (MT, ID, WY, NV, UT, CO, NM, AZ) represents 33% of the total U.S. sheep inventory (USDA-NASS, 2019) with an increasing proportion of farm flock operations that may be susceptible to GIN burden due to grazing of irrigated pastures (Bullick and Anderson, 1978). Still, estimates of predominant GIN species and related resistance to anthelmintic drug classes have not previously been evaluated in the Intermountain West. The objectives of the current study were to determine the diversity and relative levels of GIN species present, estimate the prevalence of anthelmintic resistance using the DrenchRite® larval development assay (LDA), and determine related risk factors for drug resistance from questionnaire responses.

Figure 1. Sampling locations (triangles) across MT, WY, and UT.



Materials And Methods

Sample collection

The locations of sampling sites are displayed in Fig. 1. A total of 25 sheep operations in Montana (n = 15), Wyoming (n = 9), and Utah (n = 1) were solicited to participate in the study via word of mouth communication and social media correspondence. Operations were only sampled during the summer months (June to August) to coincide with the grazing season and daily minimum temperatures adequate for GIN larval development. Breed composition varied across operations and included purebred and crossbred Targhee, Rambouillet, Polypay, Finn-Targhee, and Suffolk. To be eligible to participate producers needed to 1) operate on either irrigated or sub-irrigated (i.e., water table near the soil surface) acreage and 2) have a reported history of GIN burdens within their flocks. Once identified, co-authors traveled to each operation and obtained fecal samples of approximate equivalent quantity from a minimum of 10 ewes each displaying a FAMACHA© anemia score ≥ 3. Fecal samples were collected directly from the rectum using a gloved hand. Individual samples were composited within each operation and vacuum sealed to evacuate excess air from storage bags. Samples were then shipped overnight in a styrofoam cooler to the University of Georgia College of Veterinary Medicine (Athens, GA) for subsequent coproculture and LDA analysis.

Laboratory analyses

Upon arrival in the laboratory, the sample was thoroughly homogenized, and a fecal egg count (FEC) was performed using a modified McMasters technique (M.A.F.F., 1977). An egg isolation procedure was then performed to obtain eggs for the LDA, and a coproculture was performed to determine the genus/species of GIN present in each sample. Feces were crushed, vermiculite and water were added, and the sample was incubated at ambient temperature for 10 to 14 d. Larvae were recovered using a Baermann apparatus and identified to genus level (Dinaburg, 1942; M.A.F.F., 1977). A minimum of 100 larvae were identified unless fewer were recovered, in which case all larvae recovered were identified (Howell et al., 2008).

Larval development assays are an alternative to traditional FEC reduction tests for detecting anthelmintic resistance and were utilized in the current study due to the geographically dispersed field sampling. The maximum distance between sampled operations was ~1100 km, which represented regional diversity of sheep production, but also limited multiple visits to each location. Therefore, the LDA enabled the testing of multiple common anthelmintics at a single timepoint and reduced the logistical and financial requirements in sampling across multiple locations in the current study.

The Drenchrite LDA® was performed according to the manufacturer's recommendations and as previously described by this laboratory (Kaplan et al., 2007; Howell et al., 2008; Crook et al., 2016). Third-stage larvae (L_3) in each well were counted and identified to genus (Dinaburg, 1942; M.A.F.F., 1977). Criteria used for evaluating the resistance status of a given nematode species (*H. contortus*, *T. colubriformis*, or *Teladorsagia circumcinta*) on an operation was a minimum of 20% of that species in the control plate wells based on identification of L_3 larvae. Successful completion of the LDA required fecal samples with a sufficient number of *H. contortus* eggs present, ~500 eggs per gram (EPG).

For benzimidazoles and ivermectin, the plate well where approximately 50% of the eggs were inhibited from development to L_3 (critical well) was identified; this approximates the 50% effective concentration (EC_{50}). Ivermectin was also used to evaluate moxidectin efficacy (Kaplan et al., 2007) and the plate well where approximately 95% of the eggs were inhibited from development to L_3 (delineating well) was identified; this approximates the 95% effective concentration (EC_{95}). Thus, both the critical well and delineating well are used to estimate moxidectin resistance (Kaplan et al., 2007). It should be noted that levamisole was initially evaluated in the LDA, but its active ingredient appeared to have deteriorated in the testing plate and resulting data were not considered for the present study. Twelve operations had adequate *H. contortus* levels for resistance assessment (Year 1 = 6, Year 2 = 6), whereas only 3 operations had adequate *Trichostrongylus colubriformis* (Year 1 = 2, Year 3 = 1) and 1 operation had adequate *Teladorsagia circumcinta* for resistance assessment (Year 1).

Questionnaire

A written questionnaire on flock performance and husbandry was provided to the owner of each operation. Performance questions included number of ewes that lambed that year, average lamb crop near birth, and average weaning rate. Management systems were described by irrigation type (sub-irrigated, flood, sprinkler, or a combination) and grazing system (continuous, or rotational). Additional questions were designed to determine the previous 5 yr of commercial dewormer use by anthelmintic class (Valbazen® = benzimidazole/albendazole, Prohibit® = levamisole/nicotinic, Ivomec® = ivermectin/macrolide, and Cydectin® = moxidectin/macrolide) and manner of administration (use until ineffective, alternate products within a year, alternate products across years). Finally, producers were surveyed on usage of GIN management strategies in culling or selection (FAMACHA© anemia score, FEC, or visual appraisal).

Data analyses

Summary statistics of coproculture, LDA, and questionnaire results were calculated, and graphics created in R (R core team, 2018) and its *ggplot2* package (Wickham, 2016). Only questionnaire data from operations with successful LDA results (n = 12) were utilized to analyze the effect of producer response on anthelmintic resistance. These risk factor analyses were conducted using one-way analysis of variance in the GLM procedure of SAS (v. 9.4; SAS Institute Inc., Cary, NC), where $\log_{10}$ EC_{50} and EC_{95} values of each operation were the response variables and analyzed with questionnaire response as the explanatory variable.

Results And Discussion

Parasite identification

Sheep operations categorized by mean EPG measured in their composite fecal sample are displayed in Fig. 2. Forty four percent, 24%, 16%, and 16% had < 500, 500 to 1000, 1000 to 5000, and > 5000 EPG, respectively. Across operations, minimum, median, and maximum composite EPG were 100, 550, and 11,050, respectively. Percentage of genera of larvae identified from coprocultures of composited fecal samples at each operation are displayed in Fig. 3. Overall, *H. contortus* was most commonly identified from fecal samples across operations (average = 68.5%, minimum = 2%, and maximum = 100%) followed by *Trichostrongylus* (12.4%, 0%, and 98%,

Figure 2. Sheep operations categorized by eggs per gram (EPG) as measured in composite fecal samples (n = 25 total) and proportion of operations within each EPG class with > 50% prevalence of *H. contortus*.

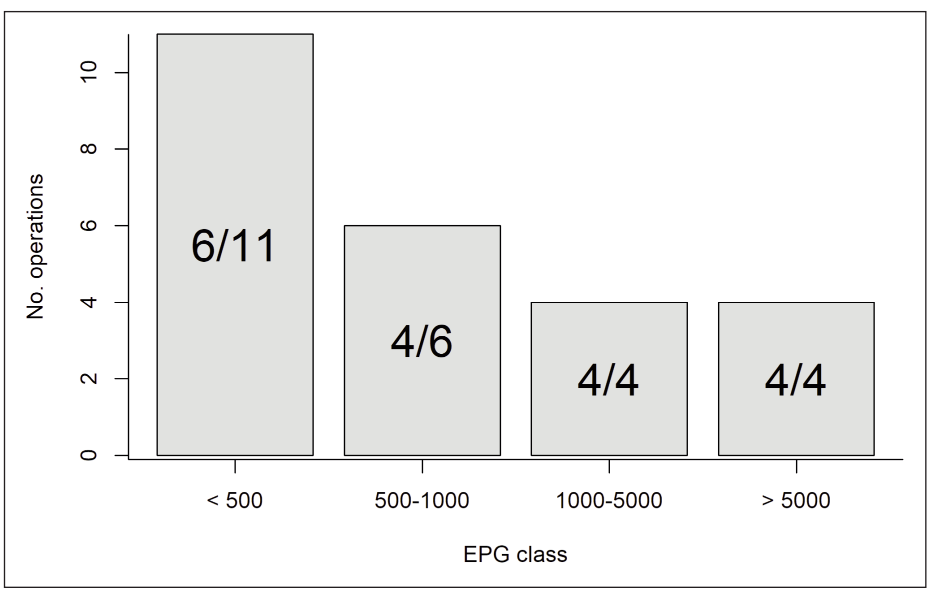
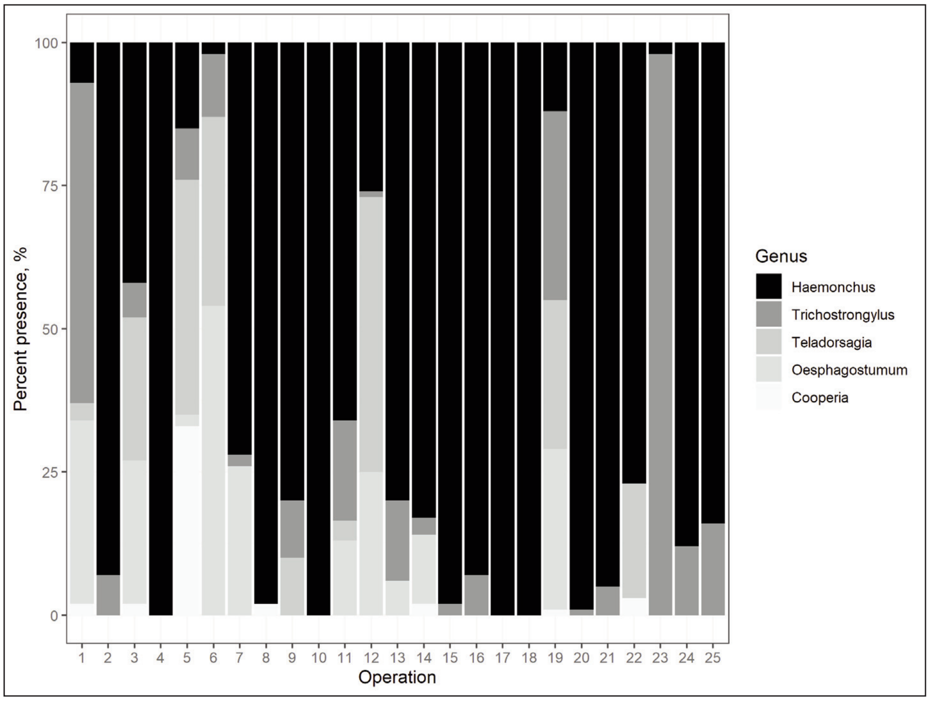


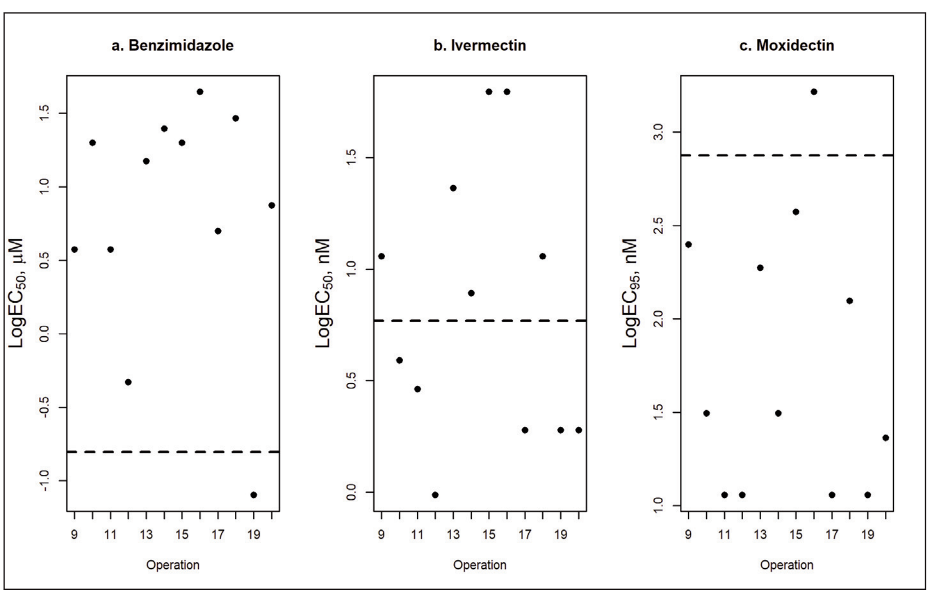
Figure 3. Percentage of the various genera identified within composited fecal samples from each sheep operation.



respectively), *Oesphagostumum* (8.9%, 0%, and 54%, respectively), *Teladorsagia* (8.4%, 0%, and 48%, respectively), and *Cooperia* (1.8%, 0%, and 33%, respectively).

Haemonchus contortus represented ≥ 50% of larvae identified from coprocultures on 18 of 25 (72%) sheep operations sampled, similar to reported results from previous field studies in the Mid-Atlantic (79%; Crook et al. 2016) and Ontario, Canada (>80%; Falzon et al., 2013a), but less than that reported from the Southeastern U.S. (96%; Howell et al. 2008). Additionally, the proportion of operations with ≥ 50% *H. contortus* increased with increasing EPG class (Fig. 2). Although *Trichostrongylus*, *Oesophagostumum*, and *Teladorsagia* represented the 2nd, 3rd, and 4th most predominant larvae identified, *Trichostrongylus* only represented ≥ 50% of larvae identified on 2 of 25 (8%) sheep operations, *Oesophagostumum* only represented ≥ 50% of larvae identified on 1 of 25 (4%) sheep operations whereas *Teladorsagia* did not exceed ≥ 50% of species composition in any operation sampled.

Figure 4. Approximate $\log_{10}$ EC_{50} and EC_{95} values for *Haemonchus contortus* from each sheep operation (n = 12) under benzimidazole (a), ivermectin (b), and moxidectin (c). The horizontal dashed line corresponds to resistance thresholds (above = resistant, below = susceptible).



Moisture availability and temperature are key determinants for GIN development into infective larvae and subsequent migration and survival. Literature reviewed by O'Connor et al. (2006) summarized necessary temperature ranges for trichostrongylid development from unembroyonated egg to L_3 as 11 to 40°C for *H. contortus*, 6 to 39°C for *Trichostrongylus* spp., and 1 to 35°C for *Teladorsagia* spp. In the current study, historic climate data near all 25 sampled operations met temperature requirements for *H. contortus* development and survival of eggs and infective larvae approximately 135 ± 17 d in a given year, highlighting limitations for *H. contortus* persistence in semi-arid continental climates of the Intermountain West (NOAA, 2020). It is possible that the wide variation in daily temperatures and lower relative humidity of the Intermountain West can be a barrier for eggs to develop into infective larvae and for newly hatched larvae to successfully develop to L_3 stages (Smith, 1990).

Utilization of irrigation systems on sampled operations clearly favored proliferation of GIN and migration of L3 larvae onto plant material even with less than optimal temperature conditions for the survival of eggs and larvae. Studies in the Intermountain West have shown greater abundance and overall survival of *H. contortus* larvae on irrigated pastures compared to non-irrigated pastures where eggs and larvae were more easily desiccated (Bullick and Anderson, 1978). Ideal deferment "rest" periods after initial grazing to minimize reinfection from L_3 GIN, while still maximizing nutritional attributes of pasture, requires additional research in the Intermountain West region.

Larval development assay

Twelve of 25 operations sampled in

the present study had parasite levels that yielded valid results for the *H. contortus* LDA. Resulting EC_{50} (benzimidazole and ivermectin) or EC_{95} (moxidectin) values for each anthelmintic class and their corresponding resistance thresholds are displayed in Fig. 4. Results indicate widespread *H. contortus* resistance to benzimidazole (92%) while fewer operations exceeded resistance thresholds for ivermectin (50%). Haemonchus contortus isolated from operation 15 exceeded the critical well but not delineating well threshold for moxidectin and would be considered low resistant (Kaplan et al., 2007; Crook et al., 2016), while operation 16 was the only one expected to be fully resistant to moxidectin. Discussion of results for *Trichostrongylus* LDA were limited to 4 operations (11, 12, 19, and 23) due to the minor proportion of this species across operations. *Trichostrongylus* larvae isolated from all 4 operations were susceptible to benzimidazole, while 2 of the 4 operations were resistant to ivermectin (11 and 19).

Estimates of *H. contortus* resistance in the current study, although limited in numbers, provide timely data from a region not previously represented in North American GIN resistance surveys (Howell et al., 2008; Falzon et al., 2013a; Crook et al., 2016). Agreement with these studies showing widespread resistance to benzimidazole is alarming especially considering > 84% of study participants had frequently utilized this anthelmintic class and were continuing to do so until informed by results of LDA. Resistance of *H. contortus* to ivermectin (50%) is similar to estimates from the Southeast and Mid-Atlantic (73 and 64%, respectively). A limitation of the current study is the lack of resistance data regarding imidazothiazoles (levamisoles) which were excluded due to assay quality concerns. Thus, additional resistance research that includes levamisole will help provide complete management recommendations to producers in the region. Resistance to moxidectin (8.3%) in the current study represents a new baseline for this region of the U.S.

Questionnaire

Questionnaire responses regarding GIN management strategies and related production parameters are summarized

Table 1. Summary of questionnaire responses on participating sheep operations.

Question/Response	No. of Respondents	Percentage, %
How is your pasture irrigated?		
Sub-Irrigated	5	31
Flood	6	38
Sprinkler	5	31
What is your grazing system?		
Continuous	7	44
Rotational	9	56
Estimated death loss due to internal parasites?		
0 to 5%	14	87.5
6 to 10%	2	12.5
11 to 15%	0	
Estimated proportion of flock performing poorly due to internal parasites?		
0 to 10%	9	56.3
11 to 20%	5	31.3
21 to 30%	1	6.2
31 to 40%	1	6.2
Do you utilize FAMACHA scoring?		
No	12	75
Yes	4	25
Did you treat with Valbazen® in the previous 5 yr?		
No	3	20
Yes	12	80
Did you treat with Safeguard® in the previous 5 yr?		
No	11	84
Yes	4	16
Did you treat with Ivomec® used in the previous 5 yr?		
No	6	40
Yes	9	60
Did you treat with Cydectin® in the previous 5 years?		
No	8	53
Yes	7	47
Did treat with Prohibit® in the previous 5 years?		
No	13	86.6
Yes	2	13.3
Do you treat with an anthelmintic product until it is ineffective?		
No	10	66.6
Yes	5	33.3
Do you alternate anthelmintic products yearly?		
No	12	75
Yes	4	25
Do you alternate anthelmintic products during the same season?		
No	11	68.7
Yes	5	31.3
Do you utilize combination anthelmintics (i.e., multiple products concurrently)?		
No	15	93.7
Yes	1	6.3

in Table 1. Unfortunately, of the 25 questionnaires distributed only 16 were returned with some questions left unanswered resulting in only 15 responses for certain questions. By study design, participating producers from smaller flocks were surveyed (average = 250 ewes, minimum = 32 ewes, maximum = 1400 ewes), with all operating in irrigated grazing systems predisposed to GIN burdens. Average lamb crop near birth and at weaning were 160% (minimum = 110%, maximum = 195%) and 146% (minimum = 105%, maximum = 180%), respectively. Perceived losses due to GIN infection are an important consideration as they can drive decision making in regard to frequency of treatment and what anthelmintic treatments are utilized. Approximately 88% of producers estimated death loss in the 0 to 5% range with 56% of producers attributing production losses to GIN in 0 to 10% of their flock. These perceptions combined with the fact that 75% of producers did not utilize targeted treatment methods (e.g., FAMACHA©, FEC) would indicate that non-target treatments at set production timepoints are common in the region.

The most commonly utilized anthelmintics on study operations over the past 5 yr were benzimidazoles including albendazole (Valbazen®) and fenbendazole (Safeguard®), 80 and 84% of respondents, respectively. Macrolides (Ivomec® and Cydectin®) were utilized by 60% and 47% of study operations, respectively. Nicotinic (Prohibit®) was the least utilized anthelmintic class over the previous 5 yr (13%). A common misconception with GIN management in the Intermountain West is the indiscriminate rotation of dewormers based on season or previous use rather than the recommended practice of switching products only when they become ineffective. Only 33% of respondents switched anthelmintic drug classes once determined inefficacious, 25% of respondents stated that they switch de-wormers yearly, and 31% reported rotating anthelmintic products within the same grazing season. Only 1 respondent (6.3%) reported using a combination anthelmintic (administration of 2, 3, or 4 anthelmintic drug classes; Kaplan 2017) which has proven to be an effective strategy internationally where widespread resistance to single anthelmintic drug classes is common. Administering combination anthelmintics has shown to reduce the development of resistance when incorporated with best management principles (e.g., refugia and FAMACHA; Leathwick et al., 2015).

Survey data has limited ability to quantify production practices on a fine-scale especially in instances where GIN management is given little thought beyond a routine yearly, whole-flock administration of dewormer. Though not surveyed in the current study, considerations such as accurate dosage based on body weight and calibration of drenching apparatus are not frequently accounted for by producers (Falzon et al., 2013b). Implementation of best management practices on a regional scale has the potential to reduce anthelmintic use and consequently GIN resistance (Learmount et al., 2016). Still, questionnaire data indicated that greater efforts to integrate Intermountain West extension and research efforts with current, research-based recommendations in regard to internal parasite management in small ruminants (e.g., American Consortium for Small Ruminant Parasite Control; *https://www.wormx.info*) are warranted.

Risk factors

As previously discussed, only 1 operation was susceptible to benzimidazoles and only 1 operation was resistant to moxidectin in the LDA for *H. contortus*. Therefore, risk factor analyses were limited to *H. contortus* response to ivermectin only (Table 2). As expected with reduced experimental units (e.g. operations with successful LDA), statistical power necessary to detect anthelmintic susceptibility differences among questionnaire responses was limited and none were significant ($P \geq 0.11$). Therefore, any numerical differences between response classes need to be taken with caution until a larger survey can be conducted.

Overuse of an anthelmintic class will promote GIN resistance more rapidly than targeted administration to the most susceptible animals. A comprehensive meta-analysis by Falzon et al. (2014) calculated ~4 times greater likelihood for resistance to anthelmintics in flocks with high frequency of treatment versus those with low frequency of treatment. Nevertheless, it's interesting to note that of the 6 operations that showed *H. contortus* resistance to ivermectin, 3 of them (9, 16, and 18) reported no past use of commercial ivermectin-containing products. Based on these results it is likely that many smaller flocks in the Intermountain West purchase replacement ewes and rams from flocks with anthelmintic resistance issues. Thus, best management practices when purchasing replacements should include at minimum inquiries of GIN burdens, his-

Table 2. Mean (± SE) $\log_{10}$ EC_{50} for the *H. contortus* LDA under ivermectin corresponding to producer responses to survey questions.

Question	Response (n)[1]	$\log_{10}$ EC_{50} Ivermectin, nM
Irrigation type	Sub (1)	-
	Flood (4)	0.63 ± 0.27
	Sprinkler (4)	1.28 ± 0.27
	Combination (2)	-
Grazing system	Continuous (5)	0.86 ± 0.27
	Rotational (6)	0.92 ± 0.25
Used Ivomec® in previous 5 yr.	Yes (7)	0.78 ± 0.22
	No (4)	1.09 ± 0.29
Used Cydectin® in previous 5 yr.	Yes (5)	1.20 ± 0.23
	No (6)	0.64 ± 0.21

[1] Means for classes with few responses (< 3) were not calculated.

tory of anthelmintic use, estimate of GIN burden (FEC), and quarantining animals upon arrival followed by appropriately treating with an effective anthelmintic.

Literature regarding effects of grazing management system on GIN treatment strategy are nuanced by regional-production dynamics (e.g., conventional vs. organic, forage species, climate, etc.; Burke et al., 2009; Colvin et al., 2012), but unique to the arid, and low precipitation Intermountain West is the ability to manage irrigation schedules to potentially manipulate larval desiccation. Still, other factors such as pasture infectivity, forage species composition, and soil moisture make management recommendations more complex than a time dependent irrigation process. Managing for forage quality while reducing conditions for GIN ingestion need to be jointly optimized. In more humid and tropical regions, recommendations have proposed a standard 3.5 d grazing period followed by a 35-d rest period (Barger et al., 1994). This strategy when tested in the south-central U.S. comparing rotational grazing to continuous grazing resulted in similar lamb weight gains, although continuous grazing required more anthelmintic intervention. Irrigated grazing systems can result in higher GIN burdens (Colvin et al., 2012), however, the ability to manipulate development of egg to the L_3 stage by delaying irrigation once sheep leave a paddock requires more research in the Intermountain West but could be an effective mitigation strategy. Integrated management strategies such as targeted selective treatment, condensed tannin-containing plants, nematode trapping fungi, and vaccines have proved effective in areas of the U.S. most affected by GIN (Terrill et al., 2012) and warrant more research and producer application in the Intermountain West.

Conclusion

Sheep managed on irrigated pasture in the Intermountain West are not exempt to *H. contortus* anthelmintic resistance issues common to the Southeastern and Mid-Atlantic regions. Consistent with published literature, *H. contortus* resistance to benzimidazole, and to a lesser extent, ivermectin and moxidectin were identified. Survey results indicated that breadth of knowledge regarding internal parasite management is limited in the Intermountain West, especially those operations dependent on irrigated pasture resources. Adoption rates of sustainable parasite control practices appear to be lacking and should be used as part of a long-term strategy to combat GIN resistance. Future longitudinal research that quantifies GIN burdens across calendar year and flock size demographics (farm vs range flocks), and the effects of grazing management in irrigated systems can refine future best management practices in the region. Still, data from this field study, although limited by number of observations, has potential to inform and adapt management of GIN in the Intermountain West.

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