PEER REVIEWED

Season-long effectiveness of stocker-calf treatment at turnout with eprinomectin extended-release injection or a combination of injectable doramectin and oral albendazole

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Abstract

On May 22, 2013, 128 stocker calves enrolled in an internal parasite control study were treated according to structured allocations and placed directly onto treatment-specific, randomly allocated, similar pastures (4 animals/2 acres (0.8 hectare) pasture). Treatment groups were saline injection (8 pastures); 0.09 mg/lb (0.2 mg/kg) BW doramectin injection concomitantly with 4.54 mg/lb (10 mg/kg) BW albendazole oral suspension (12 pastures); and 0.45 mg/lb (1 mg/kg) BW eprinomectin extended-release injection (12 pastures). Over the 119-day grazing period, average daily gains ± SE were 1.21 ± 0.07, 1.46 ± 0.04, and 1.52 ± 0.04 lb (0.55 ± 0.03, 0.66 ± 0.02, and 0.69 ± 0.02 kg)/day for the saline, combination, and extended-release groups, respectively; calves in the combination and extended-release groups outgained the control group ($P < 0.05$). In comparison to the saline-treated group, there were statistically significant ($P < 0.05$) reductions of strongyle fecal egg counts at 14, 30, and 58 days post-treatment (combination group) and at 14 and 30 days post-treatment (extended-release group). No significant differences in adult *Haemonchus placei*, *Ostertagia ostertagi*, *Cooperia punctata*, and *Oesophagostomum radiatum* populations between treatment groups were seen at necropsy. The combination treatment group had fewer *O. ostertagi* early fourth-stage larvae than did controls ($P < 0.05$). Based on results of this study, a single treatment of heavily infected and challenged stocker calves in the spring in Arkansas with either extended-release eprinomectin or doramectin + albendazole was not sufficient for adequate parasitic nematode control.

Key words: stocker cattle, nematodes, anthelmintics

Résumé

Le 22 mai 2013, 128 veaux en engraissement faisant partie d'une étude sur le contrôle des parasites internes ont été distribués de façon structurée à des pâturages similaires (4 animaux/2 acres (0.8 hectare)) choisis au hasard dans lesquels des traitements spécifiques étaient administrés. Un des traitements incluait l'injection de saline (8 pâturages), un autre l'injection de doramectine à la dose de 0.09 mg/lb (0.2 mg/kg) conjointement avec une suspension orale d'albendazole à la dose de 4.54 mg/lb (10 mg/kg) (12 pâturages) et le dernier l'injection d'éprinomectine à libération prolongée à la dose de 0.45 mg/lb (1 mg/kg) (12 pâturages). Après une période de patûrage de 119 jours, le gain moyen quotidien (erreur type) était de 1.21 ± 0.07 lb/jour (0.55 ± 0.03 kg) pour le traitement à la saline, de 1.46 ± 0.04 lb/jour (0.66 ± 0.02 kg) pour le traitement combiné et de 1.52 ± 0.04 lb/jour (0.69 ± 0.02 kg) pour le traitement avec injection à libération prolongée ($P < 0.05$). Par rapport au traitement à la saline, il y avait une réduction significative ($P < 0.05$) du compte d'œufs de strongyle dans les fèces aux jours 14, 30 et 58 suivant le traitement pour le traitement combiné et aux jours 14 et 30 suivant le traitement pour le traitement avec injection à libération prolongée. À la nécropsie, il n'y avait pas de différence significative entre les trois traitements dans la taille des populations adultes de *Haemonchus placei*, *Ostertagia ostertagi*, *Cooperia punctata* et *Oesophagostomum radiatum*. Il y avait moins de jeunes larves de quatrième stade d'*Ostertagia ostertagi* dans le traitement combiné que dans le traitement avec saline ($P < 0.05$). Chez des veaux en engraissement au printemps en Arkansas, un simple traitement avec soit une solution injectable d'éprinomectine à libération prolongée ou soit une injection combinée de doramectine et d'albendazole s'est avéré insuffisant pour un contrôle adéquat des nématodes parasitaires.

Introduction

Every phase of beef production is fraught with a multitude of considerations relative to animal health and productivity, including nematode control. The stocker/backgrounder production phase is the most challenging for nematode control, where it is often predestined for limited success. A primary challenge for effective nematode control in stocker calves is anthelmintic resistance. Generally, calves that are weaned and first enter the stocker phase of production are relatively susceptible to chemical control.[16,17,18] As calves receive repeated anthelmintic treatments and are moved from 1 grazing facility to the next, nematode burdens become an assimilation of populations of varying species and degrees of anthelmintic resistance.[4,6] A recounting of this scenario has recently been provided.[10]

Stocker/backgrounder operators are faced with several hurdles when attempting to control parasitic helminths:

- animals received are harboring worm burdens with varying degrees of anthelmintic resistance;
- no anthelmintics are available that are 100% effective against all nematode populations (no new molecular class since 1981);
- pastures in use have historically supported stocker cattle, and are often heavily contaminated with resistant helminths;
- after the receiving anthelmintic treatment, the new cattle quickly acquire new helminth infections from the pasture (nematodes previously selected for resistance) which in turn supplement the worm burdens that have survived the most recent receiving treatment;
- sustainable, non-chemical means of helminth control, such as host resistance, nutraceuticals, and grazing management, are not currently feasible or palatable at the stocker level of production;
- demand for cattle by feedlots is constant, forcing animals coming and going at the stocker level;
- there is an overriding interplay of the above with farm-specific dynamics, such as stocking rate, quality of husbandry, and parasite control, season of year (parasite transmission, hypobiosis, etc.), and parasite spectrum (nematode and trematode).

Two possible improvements over single therapeutic nematode control prior to turn-out of stocker cattle are the use of an extended-release product and treatment with a combination of albendazole and doramectin. Several treatment regimens intended to provide season-long nematode control can be formatted, such as extended-release product in combination with an unrelated chemical or repeated treatments, but extended-release products and combination therapies are the 2 treatment regimens that the cattle industry appears to favor at this time.

Materials and Methods

The methods and procedures utilized in this study were implemented to ascertain the effectiveness of anthelmintic intervention for season-long control of stocker cattle nematodiasis in the southern United States, and are consistent with the guidelines issued by the World Association for the Advancement of Veterinary Parasitology.[14] The study was conducted according to a protocol approved by the University of Arkansas Institutional Animal Care and Use Committee (Protocol #13049).

All animals were male castrates of mixed breed (primarily Angus, Charolais, Brangus, and Hereford), approximately 7 to 9 months of age and weighing 325 to 640 lb (147.4 to 290.3 kg) BW at the time of experimental treatment. All animals were obtained by a local order buyer in Hope, Ark., who assembled the cattle from regional sale outlets that received cattle from the southeastern US. Upon acquisition of the animals by the order buyer, the cattle were processed according to his standard receiving protocol which included routine (albeit not absolute) treatment with a pour-on macrocyclic lactone, usually doramectin pour-on. No study animals received anthelmintic treatment during the 14-day period immediately prior to delivery to the experiment station, which was 21 days before the study began. Immediately upon delivery to the experiment station, all animals were given uniquely numbered ear tags, and animal health and well-being assessments were conducted daily and continued until the end of the study.

From day -21 to 0, animals with poor disposition or health problems were removed from the pool of trial cattle candidates. Strongyle egg counts (eggs/gram of feces (EPG)) obtained on day -15 constituted the final exclusionary factor used for animal selection for the study. Steers with the lowest egg counts, while acceptable according to all other criteria, were removed from the pool of acceptable study cattle; day -15 counts ranged from 8 to 2205 EPG.

The study schedule is provided in Table 1. The animals arrived at the University of Arkansas Southwest Research and Extension Center in Hope, Ark, on study day -21 (May 01, 2013), and were mob-grazed across 32 study pastures until day -2, at which time they were placed on their allocated study pastures for the duration of the study. Steers received experimental treatment on day 0 (May 22, 2013) and were removed from study pastures on day 119 (Sept 18, 2013). Final body weights were obtained on days 118 and 119, and animal necropsies were performed on study days 131 to 134 (Sept 30 to Oct 03, 2013).

Body weights, fecal samples, and injection site assessments were obtained from all animals at prescribed time points throughout the study. In order to accurately determine initial and final animal body weights, animals were weighed on study days -2 and 0 (means of the 2 constituting the initial

weights), and study days 118 and 119 (means of the 2 constituting the final weights). Interim weights were recorded at approximately 30-day intervals. Animal scales (digital load cell) were independently certified immediately prior to the study, and checked for accuracy at the beginning and end of every weigh-day with multiple, certified weights.

Injection-site (right side of the neck) examinations were performed on all animals immediately prior to treatment on day 0, and at all subsequent animal handling except days 112 and 119. Fecal samples were obtained rectally as the animals were processed for weighing and injection-site assessments. Any animal void of feces at the time of collection was placed in an isolation pen for re-sampling 1 to 3 hours later, yielding a suitable sample in all but 1 occasion.

Allocation of steers to treatment group was in accordance with a generalized, randomized block design with 1-way treatment structure replicated over 2 side-by-side locations. Blocks were based on day -15 body weight and pasture location, with animals stratified within the weight blocks by coincident EPG count. Within each block, pastures were randomly assigned to 1 of 3 treatments such that each block had at least 1 pasture/treatment. Each 5 acre (2-hectare) pasture housed 4 animals, with pasture being the experimental unit. Two locations, 6 blocks, 32 replicated pastures, and 128 animals were represented in the study.

At the end of the grazing portion of the study (day 119), 1 steer from each pasture was identified for necropsy. All remaining animals were returned to the supplier after a short fecal egg count reduction study (data reported elsewhere). Selection of animals for necropsy was based on day 112 fecal egg counts (FEC), with the animal from each pasture with the log-transformed egg count closest to the pasture mean of transformed counts selected as most reflective of the co-grazed group relative to parasitism and treatment effect. Animals selected for necropsy were removed from the study site on day 119, and transported to clean, concrete-pad facilities in Savoy, Ark. All animals within a block were necropsied on the same day for parasite recovery from 12 to 15 days after grazing ceased.

The detailed design is presented in Table 2. The 3 treatment groups were: control (CON; 0.9% sodium chloride solution given by subcutaneous (SC) injection at the rate of

Table 1. Study schedule for steers enrolled in comparative anthelmintic study.

Day		
Trial day	**Calendar day (2013)**	**Event**
-21	May 01	Mob grazing on all pastures started and ended on day -2
-15	May 07	Animal body weights (BW) and fecal samples (FS)
-2	May 20	BW
0	May 22	BW, FS, injection-site examinations (ISE), and administration of experimental treatments
14	June 05	BW, FS, ISE
30	June 21	BW, FS, ISE
58	July 19	BW, FS, ISE
91	August 21	BW, FS, ISE
112	September 11	FS
118	September 17	BW, FS, ISE
119	September 18	BW
131-134	September 30 to October 03	Necropsy, ISE, FS

Table 2. Study design for the comparison of CON*, COMB†, and ERE‡ cattle over a 119-day grazing period.

Treatment group designation	Test product	Route	Dosage rate (mg/lb BW§)	Day of treatment	No. of pastures	Animals/ pasture	Animals/ treatment	No. animals necropsied at end of study
CON	Saline	SC‖	0	0	8	4	32	8
COMB	Doramectin	SC	0.09	0	12	4	48	12
	Albendazole	oral	4.54					
ERE	Eprinomectin	SC	0.45	0	12	4	48	12

*CON = control

†COMB = combination treatment with injectable doramectin (Dectomax®, Zoetis, Florham Park, NJ) + oral albendazole (Valbazen®, Zoetis, Florham Park, NJ)

‡ERE = extended-release eprinomectin (LONGRANGE™, Merial Limited, Duluth, GA)

§BW = body weight

‖SC = subcutaneous

1 mL/110 lb (1 mL/50 kg) BW); combination (COMB) doramectin[a] SC injection, given at the rate of 0.09 mg/lb (0.2 mg/kg) BW + albendazole oral suspension[b] given at the rate of 4.54 mg/lb (10 mg/kg) BW; and extended-release eprinomectin[c] (ERE) administered subcutaneously at 0.45 mg/lb (1.0 mg/kg) BW. Each injection was given on the right side of the neck immediately in front of the shoulder with a new, 16-gauge × ½-inch needle. Treatments were dosed based on day -2 body weight. All treatments were administered by 2 livestock veterinarians in a secluded area of the working facility out of view of all research station personnel, thus blinding people involved with animal care or data/fecal sample collection during the study. The 2 veterinarians that administered experimental treatments did not have any other role in the study. Data collection and laboratory work, such as FEC, coproculture counts, and nematode counts on intestinal content samples, were also done by personnel blinded to animal treatment.

Fecal samples were obtained rectally, immediately sealed, identified to animal, and placed on ice until delivery to the laboratory later the same day. Once delivered to the laboratory, the samples were refrigerated until the FEC and coprocultures were conducted (within 1 to 3 days of sample collection).

EPG counts were performed on 1-gram samples in accordance with a standard laboratory procedure (centrifugation with saturated magnesium sulfate) wherein all eggs in a 1-gram sample were floated to 1 coverslip, observed at 100X under the microscope, and counted.[15] Eggs were identified and counted as strongyle, *Nematodirus helvetianus*, *Bunostomum phlebotomum*, and *Trichuris* spp. Presence of *Moniezia benedeni* eggs and coccidial oocysts were noted. *Strongyloides papillosus* eggs were found in very low numbers, but were not counted. For each individual fecal sample with an EPG count ≥ 20 and ≥ 20 grams of feces remaining, a coproculture was constructed, subsequently harvested, and infective larvae counted in accordance with standard laboratory procedures.[5,9] The infective, third-stage larvae (L3) were identified and counted as *Cooperia oncophora*, *C. punctata*, *Ostertagia ostertagi*, *Trichostrongylus axei*, *Haemonchus* spp, and *Oesophagostomum radiatum*. All coproculture procedures and counts were performed by personnel blinded to animal number, treatment or prior counts. Only coproculture harvests which yielded a minimum of 20 L3 were used in data analysis and interpretation. For each harvest, L3 identifications and counts were limited to the first 50 larvae encountered during the random viewing (100X) of the total harvest. Species-specific percentages of the total larvae counted were determined and recorded for each sample.

Steers selected for necropsy were sacrificed by complete block at a local abattoir using captive bolt stunning followed by exsanguination, and under USDA inspection. The abomasum, small intestine, and large intestine (with cecum) were freed of mesentery, ligated *in situ*, and delivered to the research laboratory for opening, content (with wash) collection, aliquot collection, and appropriate soaking. The small and large intestine/cecum were each soaked in water at room temperature for 4 to 6 hours prior to a final abrasive wash and aliquot collection. The abomasum was soaked in water at room temperature overnight before a final abrasive wash and aliquot collection. Collected, aliquot percentages of the contents were 2% for the abomasum and 5% for the small intestine and large intestine/cecum. Collected, aliquot percentages for the soaks were 5% for the abomasum and small intestine, and 100% for the large intestine/cecum. All aliquots were washed over appropriate sieves and the backwashed residues examined (4 to 50X) for nematodes. All nematodes were identified to stage of development, genus and if possible, species.

Data were analyzed using general linear mixed models with a 2-sided, 5% level of significance.[d] Body weights and EPG counts were analyzed using repeated measures models, with the fixed effects of treatment, time point, and treatment by time point and the random effects of location, block within location, pasture within block, interaction of pasture within block, treatment and time point, and residual. Average daily gains were computed from the least squares means obtained from the body weight analysis. Counts for nematodes obtained post-necropsy were analyzed using a model with fixed effects of treatment and random effects of location, block within location, pasture, and residual. Counts directly specific to the parasitic nematodes (EPG and nematode counts obtained from necropsy samples) were log-transformed ($\log_{10}(x+1)$) prior to analysis of variance. All data are presented as back-transformed least squares means, arithmetic means, or both.

Percentage effectiveness of treatment (COMB or ERE) relative to the CON group was calculated for strongyle EPG counts at each time point during the study using the standard equation of ((C-T)/C) x 100; where C and T are back-transformed least squares means for the control group and the respective treated group.

Results

Average daily gains are presented in Table 3. Over the entire grazing period of the study (day -2 and 0 to 118 and 119), gains did not differ between the COMB and ERE treated animals, but both groups outgained the CON group ($P < 0.05$). Weigh periods were days -2, and 0 to 30, 30 to 58, 58 to 91, and 91 to 118 and 119. Steers in the ERE group gained more than those in the CON and COMB groups during the middle 2 weigh periods ($P < 0.05$). Steers in the COMB group gained more than those in the ERE group during the first weigh period, and more than CON animals for the first 2 weigh periods ($P < 0.05$). There were no significant differences in ADG among the 3 treatment groups ($P < 0.05$) during the final weigh period (day 91 to 118 and 119).

Treatment group mean animal body weights, by study day as well as over the entire grazing period, are presented in

Table 4. For study days -15 and 30, mean body weights were equivalent between all groups ($P < 0.05$). On days -2 and 0, calves in the ERE group were heavier than those in the COMB group ($P = 0.045$), but not different than the CON group. After day 58 of the study, calves in the ERE group were significantly heavier than CON animals ($P < 0.05$). Steers in the COMB group were heavier than CON animals only at the end of the study, a point in time when the weights of steers in the ERE and COMB groups were not significantly different ($P = 0.064$).

Strongyle FEC (ranges, arithmetic means with standard deviations, least squares means) by treatment group within study day, and with statistical separation between groups within day, are shown in Table 5. No egg types other than strongyle were found at levels sufficient for analysis. On study days 0, 91, 112, and 118 there were no differences in strongyle FEC between treatment groups ($P < 0.05$). On day 14, FEC were COMB < ERE < CON ($P < 0.05$). On day 30, COMB and ERE group counts did not differ and were significantly less than those in the CON group ($P < 0.05$). On day 58, ERE and CON group counts did not differ but both groups had FEC greater than found in the COMB group ($P < 0.05$). Some steers in the CON group had FEC > 1000 EPG on each sampling day. On days 14 and 30 no steers in the COMB or ERE groups had FEC > 1000, while on post-treatment days 58, 91, 112, and 118 calves in both the COMB and ERE groups were passing feces with strongyle FEC > 1000.

Mean percentages of harvested, coproculture-derived L3 by species, treatment group, and study day are presented in Table 6. An extremely low abundance of *Trichostrongylus axei* was noted, with mean percentages at harvest never > 2%. Infective larvae of *Oesophagostomum radiatum* were obtained throughout the study from animals in all treatment groups, but at mean levels ≤ 14%. *Ostertagia ostertagi* L3 were harvested from calves in all treatment groups and at each study day, with harvest percentages generally CON > COMB > ERE. Larval counts for *O. ostertagi* L3 were lowest in the middle of the study, reflective of the summer pattern of inhibition for this nematode in the south.[12] *Cooperia oncophora* L3 were harvested at high levels (32 to 36% of each treatment group's overall harvest) on day 0, but declined to only 1% for each group by the end of the study, a decline reflective of the high degree of immunogenicity conferred by this nematode.[1,2] Throughout the study, harvested L3 were predominately *C. punctata* and/or *Haemonchus* spp in all treatment groups. Harvest percentages for these 2 nematodes were similar for CON and ERE animals across all sample dates, a trend shared with the COMB group except for study day 30, at which time almost all (97%) of their L3 harvests were *C. punctata*.

Nematodes found at necropsy are listed in Table 7, along with treatment group ranges, incidences, and geometric means. Nematodes present in at least 6 control animals (minimum number infected for realistic interpretations[14])

Table 3. Average daily gains (lb, LSM ± SE) by study period and treatment group.

	Treatment group		
Study day	**CON***	**COMB†**	**ERE‡**
-2/0 to 30	0.62 ± 0.13[a]	1.06 ± 0.11[b]	0.68 ± 0.11[a]
30 to 58	1.90 ± 0.13[a]	2.17 ± 0.11[b]	2.47 ± 0.11[c]
58 to 91	1.23 ± 0.11[a]	1.37 ± 0.09[a]	1.63 ± 0.09[b]
91 to 118/119	1.15 ± 0.15	1.32 ± 0.11	1.32 ± 0.11
-2/0 to 118/119	1.21 ± 0.07[a]	1.46 ± 0.04[b]	1.52 ± 0.04[b]

[a,b,c]Means on the same line with unlike superscripts are different ($P < 0.05$)

*CON = control

†COMB = combination treatment with injectable doramectin (Dectomax®, Zoetis, Florham Park, NJ) + oral albendazole (Valbazen®, Zoetis, Florham Park, NJ)

‡ERE = extended-release eprinomectin (LONGRANGE™, Merial Limited, Duluth, GA)

Table 4. Animal body weights (lb, LSM ± SE) by study day and treatment group.

	Treatment group		
Study day	**CON***	**COMB†**	**ERE‡**
-15	465 ± 29.1	461 ± 28.9	465 ± 28.9
-2/0 average	476 ± 29.1[a,b]	472 ± 29.1[a]	483 ± 29.1[b]
30	494 ± 29.5	503 ± 29.3	503 ± 29.3
58	547 ± 29.8[a]	562 ± 29.5[a,b]	571 ± 29.5[b]
91	589 ± 30.0[a]	606 ± 29.5[a]	626 ± 29.5[b]
118/119 average	619 ± 30.2[a]	644 ± 29.8[b]	664 ± 29.8[b]

[a,b]Means on the same line with unlike superscripts are different ($P < 0.05$)

*CON = control

†COMB = combination treatment with injectable doramectin (Dectomax®, Zoetis, Florham Park, NJ) + oral albendazole (Valbazen®, Zoetis, Florham Park, NJ)

‡ERE = extended-release eprinomectin (LONGRANGE™, Merial Limited, Duluth, GA)

Table 5. Strongyle EPG* data by treatment group and day of study.

Treatment group	N	Day of study	Strongyle EPG Range	AM† (SD‡)	btLSM§
CON‖	32	0	1 – 1299	300 (281)	151
	32	14	5 – 1107	243 (264)	123[a]
	32	30	6 – 2073	312 (458)	144[a]
	32	58	0 – 2604	288 (490)	108[a]
	32	91	1 – 1890	385 (492)	150
	32	112	5 - 1965	482 (480)	220
	32	118	8 – 1968	443 (434)	220
COMB¶	48	0	4 – 2184	389 (426)	214
	48	14	0 – 51	1.4 (8)	1[c]
	48	30	0 – 738	161 (195)	36[b]
	47	58	0 – 2241	177(364)	49[b]
	48	91	0 – 2634	244 (464)	69
	48	112	1 – 2940	358 (541)	139
	47	118	0 – 2724	337 (497)	145
ERE#	48	0	5 – 1638	302 (335)	157
	48	14	0 – 438	67.1 (78)	34[b]
	48	30	2 – 406	120 (118)	59[b]
	48	58	3 – 1104	252 (270)	112[a]
	48	91	1 – 1038	279 (291)	108
	48	112	0 – 2913	468 (696)	147
	48	118	3 – 2733	481 (608)	184

[a,b,c]Back-transformed least square means of the same study day with unlike superscripts are different ($P < 0.05$)
*EPG = eggs/gram of feces
†AM = arithmetic mean
‡SD = standard deviation
§btLSM = back-transformed least square mean
‖CON = control
¶COMB = combination treatment with injectable doramectin (Dectomax®, Zoetis, Florham Park, NJ) + oral albendazole (Valbazen®, Zoetis, Florham Park, NJ)
#ERE = extended-release eprinomectin (LONGRANGE™, Merial Limited, Duluth, GA)

Table 6. Mean percentages of harvested, coproculture-derived larvae by nematode species, treatment group and study day.

Treatment group	Study day	N	*Cooperia*		*Haemonchus*	*Ostertagia*	*Oesophagostomum*	*Trichostrongylus*
			oncophora	*punctata*	spp	*ostertagi*	*radiatum*	*axei*
CON*	0	23	32	32	14	17	5	0
	14	24	8	40	25	20	6	2
	30	21	8	53	29	5	5	0
	58	19	4	26	50	15	5	1
	91	19	1	13	65	11	8	2
	118	14	1	36	40	11	13	1
COMB†	0	43	36	34	13	15	1	1
	14	1	7	36	54	2	0	1
	30	28	1	97	1	1	0	0
	58	22	2	47	47	2	2	0
	91	33	1	11	70	5	14	0
	118	35	1	30	48	14	8	0
ERE‡	0	38	33	36	16	12	2	1
	14	24	10	25	60	1	4	0
	30	25	13	37	48	1	1	0
	58	28	11	38	45	1	5	0
	91	31	5	30	61	1	5	0
	118	34	1	42	44	5	8	0

*CON = control
†COMB = combination treatment with injectable doramectin (Dectomax®, Zoetis, Florham Park, NJ) + oral albendazole (Valbazen®, Zoetis, Florham Park, NJ)
‡ERE = extended-release eprinomectin (LONGRANGE™, Merial Limited, Duluth, GA)

included adult *Haemonchus placei*, *Ostertagia ostertagi*, *O. lyrata*, *Cooperia punctata* and *Oesophagostomum radiatum*, and larval *O. radiatum* and *O. ostertagi*. All the above nematode populations, with the exception of *Ostertagia* spp EL_4, were at levels not significantly different between treatment groups ($P < 0.05$). For populations of *Ostertagia* spp EL_4, the COMB group levels were lower than CON group ($P < 0.05$).

No *Fasciola hepatica* were found in the gall bladders at necropsy, and no Fasciola-induced lesions were noted in any of the livers. No adverse reactions—injection site or animal health—to treatment were observed in this study. Additionally, no visual signs of clinical helminthiasis were noted in any of the study animals. In the course of the study, 14 animals were treated for infectious keratoconjunctivitis, 4 developed transient lameness, and 1 animal in the COMB group died of laboratory-confirmed prussic acid poisoning (Johnson grass) on study day 113. No pathologic anomalies, injection site or otherwise, were observed at necropsy.

Discussion

It is evident from this study that treating stocker cattle with either extended-release eprinomectrin injection or a doramectin + albendazole combination did not provide effective, season-long nematode control. As calculated from least squares means via the standard, FEC reduction formula with coincident treatment and CON group data, the FEC reduction percentages throughout the study are presented in

Table 7. Nematode burdens of study animals selected for necropsy at the end of the study.

	CON*			COMB†			ERE‡		
Nematode	**Incidence**	**Range**	**btLSM§**	**Incidence**	**Range**	**btLSM**	**Incidence**	**Range**	**btLSM**
Haemonchus placei									
- adult	7/8	0 – 7110	731	12/12	620 – 11600	2098	12/12	50 – 7520	1144
- L_4	2/8	0 – 500	3	5/12	0 – 500	7	8/12	0 – 290	21
Ostertagia ostertagi									
- adult	8/8	2440 – 132800	29382	12/12	9100 – 34800	16860	12/12	2470 – 49120	11849
O lyrata									
- adult	8/8	80 – 1840	411	7/12	0 – 1800	41	8/12	0 – 1540	47
Ostertagia spp – larvae									
- LL_4	8/8	1480 – 27600	4794	12/12	740 – 5280	1922	12/12	190 – 12740	2846
- EL_4	8/8	350 – 25800	1676[a]	12/12	80 – 2740	331[b]	12/12	40 – 6920	591[ab]
Trichostrongylus axei									
- adult	3/8	0 – 300	5	1/12	0 – 120	<1	1/12	0 – 20	<1
T. colubriformis									
- adult	3/8	0 – 120	4	3/12	0 – 250	2	3/12	0 – 400	3
Cooperia adult									
-*oncophora*	2/8	0 – 1410	4	2/12	0 – 100	1	7/12	0 – 3900	47
-*punctata*	8/8	150 – 23680	1943	12/12	150 – 27020	1970	12/12	100 – 47460	6225
Oesophagostomum radiatum									
- adult	7/8	0 – 372	16	11/12	0 – 875	99	12/12	21 – 524	99
- L_4	6/8	0 – 27	4	5/12	0 – 23	2	6/12	0 – 21	1
Trichuris spp									
- adult	0/8	0 - 0	0	1/12	0 – 83	<1	0/12	0 - 0	0

[a,b]Geometric means (btLSM) on the same line with unlike superscripts are different ($P < 0.05$)

*CON = control

†COMB = combination treatment with injectable doramectin (Dectomax®, Zoetis, Florham Park, NJ) + oral albendazole (Valbazen®, Zoetis, Florham Park, NJ)

‡ERE = extended-release eprinomectin (LONGRANGE™, Merial Limited, Duluth, GA)

§btLSM = back-transformed least square mean

Figure 1. With 90% reduction in FEC the minimal standard for a nematocidal treatment to be considered efficacious,[11] only the COMB treatment at post-treatment day 14 was efficacious. For the remainder of the study, there was a steady decline in EPG reductions for the COMB group. Reductions in FEC for the ERE group were 72% on day 14 and 33% on day 112, points in time roughly coinciding with the 2 accelerated release times of eprinomectin from the depot matrix at the injection site.[7,13]

In addition to interpretations made from the strongyle FEC and their treatment-specific reductions, coproculture data provided insight as to which strongyle species were maintaining and/or initiating patencies during the "efficacious" periods of drug therapy. No strongyle species burdens were rendered non-fecund by any treatment regimen. Clearly, fecundities for all strongyle species were significantly curtailed during the periods when FEC were significantly reduced, up to day 30 for the ERE group and up to day 58 for the COMB group. Beyond those time points, coproculture and FEC data indicate that all the strongyle burdens were as fecund in the treated animals as they were in the controls. *O. ostertagi* is an apparent exception to the above, as this nematode does appear to have been inhibited from normal rates of fecundity at all post-treatment points in the ERE group.

Coprology data, as discussed above, can only provide indirect evidence of worm burden sizes, per-worm fecundities, and drug efficacies. Necropsy of sentinel animals from each treatment group would have to be done in order to obtain absolute documentation of worm burden, fecundities, and drug-efficacy at time points during the study. Animal necropsy in this study was only conducted with animals removed from pasture at 119 days post-treatment, a point in time when no efficacious (> 90%) FEC reductions were detectable, regardless of treatment group. At necropsy, mean numbers of adult *H. placei*, adult *C. punctata*, and adult *O. radiatum* were higher for both anthelmintic treatment groups than for the CON group. Reductions were seen for both anthelmintic groups in respect to all *O. ostertagi* (including *O. lyrata*) populations (adults, EL4 and LL4), but none of the reductions were greater than 80%.

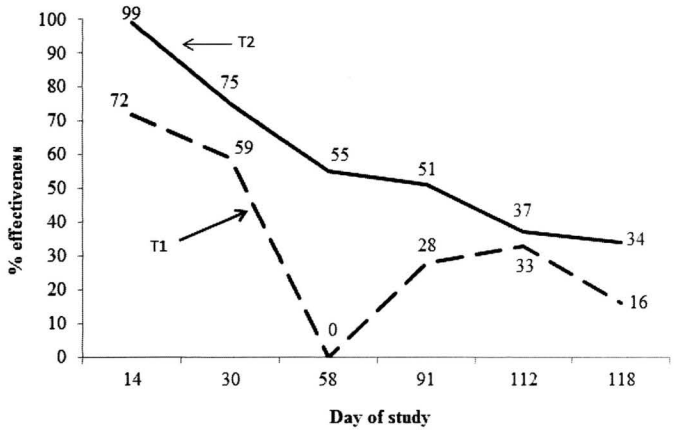

Figure 1. Fecal egg count reduction (FECR) percentages by day of study, with treatment groups being CON (T3), COMB (T2), and ERE (T1), and utilizing same-day, back-transformed least squares means by standard equation.*

$$*\text{FECR \%} = \left[\frac{\text{day}_x\ \text{T1 or T2} - \text{day}_x\ \text{T3}}{\text{day}_x\ \text{T3}}\right] \text{X } 100$$

†CON = saline injected control

‡COMB = combination treatment with injectable doramectin (Dectomax®, Zoetis, Florham Park, NJ) + oral albendazole (Valbazen®, Zoetis, Florham Park, NJ)

‡ERE = extended-release eprinomectin (LONGRANGE™, Merial Limited, Duluth, GA)

Despite the lack of season-long nematocidal efficacy, weight gains of steers were significantly higher in the ERE and COMB groups than in CON steers, with an average 36.6 lb (16.6 kg) and 28.9 lb (13.1 kg), respectively, more weight gain.

The current study was a parasitological "perfect storm", wherein light-weight stocker steers carrying both heavy and resistant nematode burdens were treated, and then placed onto pastures heavily seeded with more resistant nematodes. It is doubtful that any avermectin would have been effective in this study for either the short- or long-term, regardless of formulation or combination. Avermectin resistance has long been accepted as the norm in cattle nematodes,[8] resistance which is most demonstrable at the stocker/backgrounder level.[17,18] Timely treatment of cattle with a benzimidazole might serve to eliminate ML-resistant nematode populations, but benzimidazoles have limited efficacy against inhibited nematodes,[16] plus they possess no residual nematocidal properties.

The standing recommendation in Arkansas is to treat stocker calves at arrival processing with an anthelmintic (or combination) followed by periodic FEC to document effectiveness of the anthelmintic treatment, as well as to monitor the change in egg counts to "significant levels". In the current study, implementation of these recommendations would have translated into 1) benzimidazole treatments being given to the cattle treated with ERE soon after it was obvious that the ERE treatment was not efficacious, and 2) repeat of the COMB treatment approximately halfway through the grazing period. Forbes recommended that an unrelated product (benzimidazole or imidazothiazole) be administered with ERE if elevated nematode FEC levels persist post-treatment;[7] this recommendation can be found on the ERE package insert. This recommendation has been made in respect to propagation of resistant populations at the expense of *refugia*, and not necessarily for the provision of efficacious treatment, the latter being an assumed property which was not evident in the current study.

Effective chemical control of nematode populations in small ruminants is currently the exception rather than the rule. Unfortunately, this same situation is becoming common with stocker cattle. Within the confines of routinely assessing

anthelmintic efficacies (i.e., FEC reduction tests, percentages, statistics), most anthelmintic treatments for stocker cattle today are of limited efficacy, with cattle resilience and resistance shouldering more and more of nematode control. To be sure, this resilience and resistance, factors that entail protein loss, energy loss, and decreased feed efficiency, comes at a loss to animal well-being and productivity. The statement "the time is right" for new anthelmintics for livestock was overdue when it was made nearly a decade ago.[3] No new anthelmintics appear positioned for imminent commercial availability for use in cattle in the US. As such, we are relegated to evaluating and strategically utilizing currently available products, actions that will provide for the best realistic nematode control today, as well as provide additional useful life for the chemicals we have.

Conclusions

In the stocker industry, extended-release treatments must be scrutinized for propagation of resistant populations. Additionally, anthelmintic combinations must be strategically administered and targeted, as well as scrutinized for selection of multi-class resistance. Hopefully, combination products, given their need in the cattle industry, might someday be formulated for use in the US.

Endnotes

[a]Dectomax® Injectable Solution, Zoetis, Florham Park, NJ
[b]Valbazen® Suspension, Zoetis, Florham, NJ
[c]LongRange™, Merial, Duluth, GA
[d]SAS version 9.3, SAS Software Inc., Cary, NC

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