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Gastrointestinal parasitic worm burdens and efficacy of deworming practices in growing beef cattle grazing California pastures

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Abstract

Treatment for enteric parasites is a common practice in beef cattle, yet little data is known about the prevalence of nematode and trematode parasite infections in beef cattle in the western United States. Likewise, the data on the efficacy of deworming practices and the presence of anthelmintic resistance (AR) of these parasites in this region is sparse. The current study collected evidence for the presence of nematode and trematode parasites in 18 herds of young beef cattle grazing either dryland or irrigated pasture in northern California as well as on efficacy and evidence of AR in a subgroup of herds. We found variable levels of fecal egg counts (FEC) ranging from 6 to 322 for the arithmetic mean eggs per gram (EPG) in the tested cattle groups. There was no difference in the number of EPG between herds grazing dryland or irrigated pasture ($P = 0.54$). We did not find any evidence for liver flukes or lungworms in the tested cattle. There was evidence of AR to macrocyclic lactones in all eight herds where fecal egg count reduction tests (FECRT) were performed, however due to types and execution of treatment applications and sample sizes, these results need to be interpreted with caution. The most common genus of third stage larvae in coproculture testing before treatment was *Cooperia* (between 55% and 98% of larvae) as well as post treatment for those herds undergoing FECRT (between 50% and 96%). *Ostertagia* was the second most frequent genus of larvae found in coproculture testing making up between 0% and 27% of larvae before treatment and between 5% and 50% of larvae after treatment. Anthelmintic practices in beef herds in northern California and likely in a larger geographic area in the western United States need to be updated in order to continue effective use of the currently available drugs.

Lay Summary

Beef cattle are routinely treated for intestinal parasites with drugs labeled for this purpose, but little data are available on how common infections with these parasites are in beef cattle in the western United States. There is also sparse data on how much resistance to the available drugs exists in intestinal parasites in this region. The current study collected data on evidence for the presence of roundworm and liver flukes in 18 herds of young beef cattle grazing dryland or irrigated pasture in northern California as well as how effective currently used drugs are in killing them. Mean parasite eggs per gram (EPG) per herd tested in fecal samples ranged from 6 to 322, while no liver fluke or lungworm eggs were detected. There was no difference between EPG in cattle grazing dryland or irrigated pasture. The class of dewormer used was not effective in killing the parasites to the degree expected. Testing of larvae in fecal samples showed that one genus of worm, *Cooperia*, was most common before and after treatment, followed by the genus *Ostertagia*. The results show that deworming practices in northern California herds and likely the larger geographic area in the western United States need updating.

Key words: anthelmintics, anthelmintic resistance, beef cattle, gastrointestinal parasites, nematode parasites, trematode parasites

INTRODUCTION

Treatment for intestinal parasites is commonly practiced in grazing beef cattle operations as part of herd health management and to increase efficiency of the herd. Among the perceived benefits of anthelmintic treatment are improved immune status (Wiggin CJ, 1989), increased weight gain (Ciordia et al., 1984; Wohlgemuth K, 1988) and better reproductive performance (Stuedemann et al., 1989). Today, as has been the case for the last thirty years, there are three primary classes of anthelmintics available for the treatment of beef cattle in the US, the benzimidazoles, the imidazothiazoles/tetrahydropyrimidines, and the macrocyclic lactones (ML). No new anthelmintic class or specific drug has been approved by the Food and Drug Administration (FDA) in many years and the industry relies on the continued efficacy of the available drugs. Anthelmintic resistance (AR) in cattle has already been documented in other geographic areas of the United States (Gasbarre, 2014) and other countries (Geurden et

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al., 2015; Kelleher et al., 2020). However, there is a paucity of information on intestinal parasite burdens and the presence of AR in the western United States and in particular in California, with a mediterranean climate with cool, moist winters and hot, dry summers (Macon, 2016). California has a sizeable beef production sector ranking 15th in the nation with 680,000 beef cows (United States Agricultural Statistics Service, 2022). The objective of this study was to characterize anthelmintic drug use and the parasitic burden of growing beef cattle grazed on dry and irrigated pastures in California, and to assess the efficacy of anthelmintic drugs used in this population. The study hypotheses were that ML drugs are most commonly used, cattle grazed on irrigated pastures have higher parasite burdens than those grazed on dryland, and that there is evidence of AR in California beef herds as assessed through a fecal egg count reduction test (FECRT) as has been observed elsewhere.

MATERIALS AND METHODS

The animal procedures in this study have been reviewed by the Institutional Animal Care and Use Committee under protocol #22201 at the University of California, Davis. This institution has an animal welfare assurance on file with the Office of Laboratory Animal Welfare (OLAW). The Assurance Number is D16-00272 (A3433-01).

Upon review of the study protocol, the institutional review board at the University of California, Davis granted an exemption for use of a questionnaire submitted to participating cattle ranchers (IRB-ID 1736274-1).

Recruitment of Farms

The study took place between March and August 2021. A convenience sample of ranches where the owner was known to one of the authors and was willing to participate in the study was recruited through a network of livestock advisors within the University of California's Agriculture and Natural Resources system. Enrollment criteria were pasture exposure during the previous six months and no treatment with anthelmintics in the 45 d prior to the visit, or not within the previous 150 d with long-acting eprinomectin. *Bos taurus* cattle of either sex between 6 and 18 months of age were eligible to be enrolled as the group suspected to be most susceptible and affected by intestinal parasite infestation (Claerebout and Vercruysse, 2000). Herds with a history of grazing either dryland or irrigated pasture were enrolled. Herds were visited at a time when their owners or managers had scheduled anthelmintic treatment in calves of the target age and producers used the product of their choice in the manner they typically employ. A short questionnaire was used to capture information on herd size, date and name of the last anthelmintic used as well as which dewormer they were using on the day of enrollment and the route of administration. Additional questions included how the dose per animal was determined, the pasture type (irrigated or dryland) cattle had been grazing before treatment, whether producers usually treat cattle for liver flukes and with what drug, how often they use a dewormer in preweaned and weaned calves and adult cattle and which products they use for these respective treatments.

Fecal Testing and Fecal Egg Count Reduction Test

In all participating herds, a target of 10 animals (Coles, et al., 1992; Maurizio et al., 2021; Sabatini et al., 2023) had an individual fecal sample taken for fecal egg count (FEC) testing. To assess the efficacy of the deworming process, a FECRT was performed on herds able to present to us the same calves again two weeks after deworming. Some additional calves, between 5 and 10 per herd, included in the initial sampling were originally intended as negative controls and were not treated until after the second sampling. However, according to the latest recommendations on FECRT we did not use the negative controls in the FECRT analysis (Kaplan et al., 2023) but included them in the initial FEC. The FECRT evaluates the reduction in fecal eggs approximately 14 d after treatment depending on the anthelmintic drug used. Specific recommendations according to the 1992 World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines, which were still in place at time of sample collection, were to collect follow-up samples 10 to 14 d for after the initial sample (Coles et al., 1992). Updated WAAVP guidelines from 2023 for follow-up sampling are now between 10 to 14 d for levamisole and benzimidazoles, 14 to 17 d for macrocyclic lactones, 17 to 21 d for moxidectin, and 21 to 28 d for long-acting macrocyclic lactones with the caveat that there is still insufficient data to define the optimal time point for these long-acting drugs (Kaplan et al., 2023). In a susceptible parasite population, the lower limit 90% confidence interval (CI) for efficacy is ≥90% for clinical or ≥95% for research protocols and the upper 90% CI for efficacy is ≥99%. Resistant parasite populations will result in efficacies where the upper 90% CI is less than 99%. An inconclusive result is where the upper 90% CI is ≥99%, but the lower 90% CI is <90% for clinical or <95% for research protocols (Kaplan et al., 2023). About 50 g of feces were collected from the rectum of a target sample of ten individual animals using a fresh palpation sleeve for each calf. Samples were stored in individual sterile plastic bags and stored on ice before delivery to the lab the next day. Samples were screened for the presence of trichostrongylid eggs via the Mini FLOTAC technique (Cringoli et al., 2017). The Mini FLOTAC system has a sensitivity of 5 eggs per gram (EPG) and is considered an accurate and precise method for visual diagnosis of protozoan and helminth infections in animals (Cringoli et al., 2017).

Prior to further processing at the lab, sample bags were manually massaged to help homogenize distribution of fecal eggs. An aliquot of 5 g of feces was measured and placed into the Fill-FLOTAC before adding a saturated saline flotation solution with a specific gravity of 1.20 for a total volume of 45 mL. The fecal suspension was homogenized by pumping the homogenizer up and down followed by twisting10 times. Before filling the Mini FLOTAC reading discs, the Fill-FLOTAC was inverted 5 times. After a rest period of 10 min, each of the two chambers was viewed under 100x magnification via microscope and inspected for the presence of trichostrongylid eggs. Once eggs were counted, EPG was calculated by adding the counts of both chambers and using a 1:5 dilution factor. Those samples with insufficient fecal matter or if a sample could not be obtained from an animal during the second sampling, the animal was omitted from the data set for the FECRT.

Coproculture testing for third stage larval identification was performed on a homogenized composite sample from all calves for herds tested once only or all treated calves for herds undergoing FECRT. At least 50 g total fecal matter per herd was shipped to the Texas A&M parasitology laboratory to determine the percent of each species of roundworm larvae in

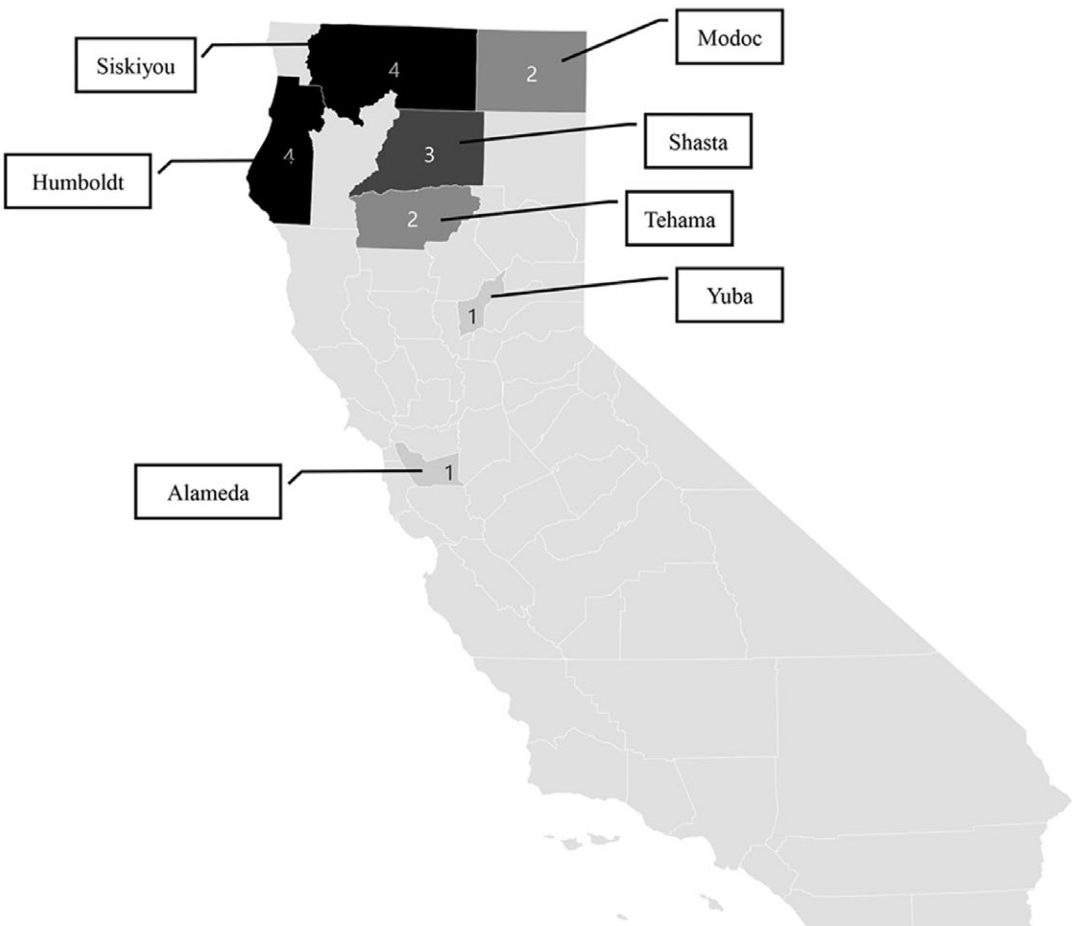

Figure 1. Counties and number of herds grazing per county in a study on intestinal parasite burden and anthelmintic resistance in Northern California between March and August 2021.

a sample. For herds undergoing FECRT, samples from both collection days were submitted.

A further portion of at least 10 g of a composite fecal sample per herd was submitted to the California Animal Health & Food Safety (CAHFS) lab for Baermann and fecal sedimentation testing for the presence of lungs worms and liver flukes respectively.

Statistical Analysis

Arithmetic means of fecal EPG for each herd were calculated with the formula $\bar{x} = \frac{x_1 + x_2 + x_3 \ldots x_n}{n}$ while the geometric means were calculated with the formula $G.M. = \sqrt[n]{(x_1 * x_2 * x_3 \ldots x_{1n})}$ where $x_1 - x_n$ are the individual results for EPG determined by the Mini FLOTAC assay and n is the number of individuals in the group.

Egg counts were compared between groups grazing irrigated pasture and dryland in SAS v. 9.4 (SAS Institute, Cary, NC) with animal as the unit of analysis in a negative binomial mixed model with herd as the random effect, pasture type (dryland or irrigated pasture) as the predictor and calf age in months and prior deworming status (yes/no) as covariates. Significance level was set at $P < 0.05$.

Efficacy of the anthelmintic drugs was evaluated using the online web application "Modelling Faecal Egg Counts with Shiny" (http://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/) (Torgerson et al., 2014), which utilizes Bayesian hierarchical modeling and using the individual animal as the unit of analysis. Analysis for paired samples with individual efficacy was chosen, which models different treatment efficacies for each animal (Wang et al., 2018). A confidence level of 0.1 was selected resulting in 90% confidence intervals as described in the WAAVP guidelines (Kaplan et al., 2023).

RESULTS

Demographics and Anthelmintic Use

Eighteen farms met eligibility criteria of pasture exposure of *Bos taurus* cattle of either sex between 6 and 18 mo of age during the previous six months and no treatment with anthelmintics in the 45 d prior to the visit, or not within the previous 150 d with long-acting eprinomectin. Herds grazed in seven different counties as follows: one in Alameda, four in Humboldt, two in Modoc, three in Shasta, four in Siskiyou, two in Tehama, and one in Yuba county (see Figure 1). Climate and landscapes are variable between counties where cattle were grazing with colder winters in mountainous Siskiyou and Modoc counties compared to counties further south or at lower elevation. Coastal counties Humboldt and Alameda enjoy cooler summers compared to inland counties (California Department of Fish and Wildlife, 2021). Eleven herds had grazed on dryland and seven on irrigated pasture. Herds on dryland pasture consisted of herd sizes between

<100 to >500 head while herds on irrigated pasture ranged from between 100 to 249 to >500 per herd (Table 1). Two herds (herd D5 and I7) were certified organic that do not use routine anthelmintic drug treatments. Calves were between six and seventeen months old and included both steers and heifers. In addition to the two organic herds, two other herds had not dewormed enrolled calves previously. All other calves had been dewormed at least 77 d prior to enrollment. Of the fourteen herds that had dewormed previously, three herds reported prior benzimidazole use, while eight reported prior ML use, and three were unsure about the drug used prior in the enrolled calves. Frequency of routine deworming in non-organic herds was reported as follows: for preweaned calves, two herds do not deworm, nine herds deworm once, four herds deworm twice, and one herd more than twice; for weaned calves, six reported one time deworming, three deworm twice, four herds more than twice, and three herds did not report; for adult cattle, one herd reported no deworming, four herds reported once a year, six herds twice a year, one herd more than twice a year, one herd gave the answer "sometimes', two did not answer, and one herd was uncertain. The administration of drugs used during the trial by each farm was either through pour-on application or subcutaneous injections, and all used the same ML class anthelmintic except for the organic herds, which did not use any anthelmintics. Four ranches reported regular flukicide use in their herds. Three producers administered doses based on the estimated weight of individual animals while 11 administered the same dose for all the calves in their herd and two measured the weight of small groups between 5 to 10 animals prior to dosing. For single sampling, 148 calves were sampled, while 73 calves were available for FECRTs.

Initial Sampling Results All Herds

Results for initial sampling of all herds are shown in Table 2. Arithmetic mean EPG ranged between 6 and 322, while geometric mean EPG ranged between 1 and 174. The last dewormer used in these cattle was in the benzimidazole class for three of the groups and in the ML class for eight of the groups. Four groups had not been dewormed prior and three producers could not recall or did not know the last dewormer used. All fecal sedimentation and Baerman test results were negative. Coproculture results for ten herds not participating in FECRT are reported in Table 3. *Cooperia* appeared as the dominant genus in all samples, while seven herds had larvae belonging to the genus *Ostertagia* that made up above 10% of larvae hatching from the composite sample. One of these herds also had 14% of larvae belonging to the genus *Haemonchus*, one of only three samples among this group where larvae from this genus were identified. *Osophagostomum* and *Trichostrongylus* larvae were rare or absent in all samples, consisting of a maximum of 14% of hatched larvae.

Comparison of Egg Counts by Pasture Type

There was no statistically significant difference between egg counts from calves on irrigated pasture versus those on dryland ($P = 0.54$) in the model. The covariates average reported calf age in months ($P = 0.02$) as well as whether the group had been previously dewormed ($P = 0.03$) were statistically significant predictors of EPG. Calves are expected to have lower egg counts by a factor of 1.28 for each month of life holding other predictors constant and those that were not previously dewormed are expected to have lower egg counts by a factor of 3.22 than those that have previously been dewormed holding other predictors constant. Least square means egg count estimates for dryland and irrigated pasture were 38 (±11.1) and 48 (±16.8) EPG respectively. Model estimates are reported in Table 4.

Fecal Egg Count Reduction Testing

Results for FECRT are presented in Table 5. All calves undergoing FECRT were treated with an anthelmintic belonging to the ML drug class. Two groups received a dewormer with a pour-on application (I2 and D10) while all others received injectable dewormers. pretreatment egg counts for individual calves ranged between 0 EPG and 1305 EPG as reported in Table 2. Mean EPG pretreatment as determined by the Bayesian hierarchical modeling for enrolled groups ranged between 19 and 293. Post-treatment EPG for individual calves ranged between 0 and 580 and model estimated mean EPG for groups were between 2 and 147. The percent of fecal egg count reduction achieved was between 44% (90% CI: 23% to 69%) and 97% (90% CI: 89 to 100). Therefore, all the herds could be classified as resistant according to the guidelines for both clinical or research protocols.

Coproculture of composite samples resulted in a similar distribution of genera than in the herds sampled only once (Table 6). *Cooperia* was the dominant genus of larvae hatching from fecal eggs both pre and post treatment in the participating herds, making up between 69% and 94% of the larvae. Likewise, the second most common genus was *Ostertagia*, contributing between 0% and 27% of the larvae hatching from eggs in the fecal samples. *Haemonchus, Oesophagostomum*, and *Trichostrongylus* were identified to a much lesser degree in the samples.

DISCUSSION

The present study provides an update on the prevalence of gastrointestinal parasites in northern California beef herds as well as the usage patterns of anthelmintic drugs by their producers. Individual fecal egg counts varied widely in individuals between 0 and 1305 EGP. Geometric means of egg counts per tested herds varied between 1 and 174. The study also provides evidence for the widespread resistance of these parasites to ML, the most commonly used anthelmintic drug class, a concerning trend which has been observed in other parts of the United States as well as other parts of the world. However, some caution in the interpretation of the FECRT is warranted as explained below.

Parasite Prevalence

Participating herds are part of the network of producers known to the University of California Cooperative Extension advisors participating in this study. As such, they may represent a biased sample and not necessarily be representative of all cattle producers in the state. Participants may have had a particular interest in parasite control, or they may have suspected AR in their herds already. However, a mix of herd sizes, dryland and irrigated pasture grazing, as well as a variety of locations and landscapes where cattle were grazing was represented in the sample. Herds grazing on dryland were often not able to participate in the FECRT because of the extensive terrain cattle are grazing and gathering them twice within a 2-week period would have been an inconvenience for their owners. Contrary to our hypothesis, not all cattle

Table 1. Herd size and anthelmintic use of beef herds participating in a study on intestinal parasite burden and anthelmintic resistance in Northern California between March and August 2021

Item	Dryland, *n* = 11	Irrigated pasture, n = 7
Herd Size Category		
<100	4	0
100-249	3	2
250-499	3	1
500+	1	4
Use of same dewormer than previous dewormer in this group of calves		
Yes	5	1
No	3	2
None used before	2	2
Unknown	1	2
Active ingredient of last dewormer used		
Benzimidazoles		
Albendazole	1	0
Fenbendazole	1	1
Macrocyclic Lactones		
Doramectin	3	0
Eprinomectin	1	0
Ivermectin	2	1
Moxidectin	0	1
Unknown	1	2
None	2	2
Active ingredient current use		
Benzimidazoles		
Albendazole	0	0
Fenbendazole	0	0
Macrocyclic Lactones		
Doramectin	5	1
Eprinomectin	2	3
Ivermectin	3	2
Moxidectin	0	0
None	1	1
Drug class current use		
Macrocyclic lactone	10	6
Benzimidazole	0	0
None	1	1
Administration route current use		
Injectable	7	5
Pour-on	3	1
None	1	1
Routine flukicide use in herd		
Clorsulon	1	1
Albendazole	1	0
None	8	6
Unknown	1	0
Dose determined per animal		
Estimate individual weight	3	0
Same dose for all	6	5
Individual weight measured	0	0
Group weight measured	1	1
No anthelmintic use	1	1

Table 2. Results of fecal testing for gastrointestinal parasites for herds undergoing single sampling during a study on intestinal parasite burden and anthelmintic resistance in Northern California between March and August 2021

Herd	Pasture type	Herd size	Number of calves tested	Age of calves in months	Arithmetic mean EPG	Geometric mean EPG	Range EPG	Last dewormer active ingredient, drug class and formulation	Fecal Sedimentation	Baermann
D1	Dryland	100-249	10	10	215	87	5-800	Doramectin, Macrocyclic lactone, injectable	Negative	Negative
D2	Dryland	<100	10	12	62	30	0-200	Eprinomectin, Macrocyclic lactone, injectable	Negative	Negative
D3	Dryland	250-499	10	17	7	1	0-20	Ivermectin, Macrocyclic lactone, pour-on	Negative	Negative
D4	Dryland	<100	10	13-15	322	174	25-1175	Ivermectin, Macrocyclic lactone, pour-on	Negative	Negative
D5	Dryland	100-249	11	15	6	2	0-15	None	Negative	Negative
D6	Dryland	<100	13	11	63	54	15-115	Fenbendazole, Benzimidazole, oral	Negative	Negative
D7	Dryland	>500	14	12	124	69	25 - 630	Doramectin, Macrocyclic lactone, injectable	Negative	Negative
D8	Dryland	250-499	10	10	203	90	20 – 1305	Unknown	Negative	Negative
D9	Dryland	250-499	8	10	186	136	35 – 415	Albendazole Benzimidazole, oral	Negative	Negative
D10	Dryland	<100	20	10	56	26	0 - 185	Doramectin, Macrocyclic lactone, pour-on	Negative	Negative
D11	Dryland	<100	14	7-9	18	5	0-80	None	Negative	Negative
I1	Irrigated	100-249	15	10	154	64	0 - 525	Ivermectin, Macrocyclic lactone, injectable	Negative	Negative
I2	Irrigated	>500	15	12-13	25	4	0 - 100	None	Negative	Negative
I3	Irrigated	>500	10	12	72	46	20 - 250	Fenbendazole, Benzimidazole, medicated feed	Negative	Negative
I4	Irrigated	250-499	13	12	23	15	5-65	Unknown	Negative	Negative
I5	Irrigated	>500	13	12	58	36	10 - 160	Unknown	Negative	Negative
I6	Irrigated	>500	15	9-10	58	16	0 - 160	Moxidectin, Macrocyclic lactone, pour-on	Negative	Negative
I7	Irrigated	100-249	10	11	15	6	0-30	None	Negative	Negative

Table 3. Coproculture results from fecal testing for herds undergoing single sampling during a study on intestinal parasite burden and anthelmintic resistance in Northern California between March and August 2021

Herd ID	No. of L3 larvae	%Cooperia	%Haemonchus	%Ostertagia	%Oesophagostum	%Trichostrongylus
D1	348	98	1	1	1	0
D2	142	82	0	12	0	6
D3	44	75	14	11	0	0
D4	62	95	0	2	0	3
D5	0	NA	NA	NA	NA	NA
D6	133	89	0	14	0	0
D9	140	84	0	14	1	0
D11	74	73	3	22	3	0
I4	29	55	0	24	7	14
I7	120	75	0	23	2	0

Table 4. Negative binomial model to estimate the effect of dry or irrigated pasture on the outcome eggs per gram in groups of cattle between the age of 8 and 17 months of age in 18 northern California beef herds

Variable	Beta	Standard error	*P*-value
Intercept	7.31	1.25	< 0.01
Pasture			
Irrigated	Reference		
Dry	−0.22	0.36	0.54
Age in months	−0.25	0.11	0.02
Prior dewormed			
Yes	Reference		
No	−1.17	0.53	0.03

Table 5. Fecal egg count reduction test during a study on intestinal parasite burden and anthelmintic resistance in Northern California between March and August 2021

Herd ID	No. of calves	Pretreatment mean EPG (90% CI)	Post treatment EPG range	Post treatment mean EPG (90% CI)	%FECR (90% CI)	Sampling interval days	Active Ingredient and drug class and formulation
D7	9	142 (82 – 305)	5 – 160	60 (27 – 149)	57 (32 – 77)	16	Doramectin, macrocyclic lactone, injectable
D8	8	293 (155 – 708)	15 – 580	147 (58 – 381)	48 (27 – 75)	17	Eprinomectin, macrocyclic lactone, injectable
D10	9	63 (34 – 160)	0 – 35	15 (6 – 46)	76 (56 – 88)	13	Doramectin, macrocyclic lactone, pour-on
I1	9	189 (95 – 506)	0 – 340	103 (42 – 291)	44 (23 – 69)	16	Ivermectin, macrocyclic lactone, injectable
I2	10	19 (11 – 37)	0 – 20	6 (2 – 14)	69 (39 – 85)	14	Ivermectin, macrocyclic lactone, pour-on
I3	10	80 (49 – 149)	0 – 100	33 (14 – 72)	58 (33 – 81)	14	Doramectin, macrocyclic lactone, injectable
I5	8	50 (28 – 112)	0 – 55	23 (9 – 57)	54 (27 – 78)	19	Eprinomectin, macrocyclic lactone, injectable
I6	10	70 (35 – 203)	0 – 5	2 (0 – 11)	97 (89 – 100)	13	Eprinomectin, macrocyclic lactone, injectable

herds grazing dryland had low FEC, and there was no difference in the estimated EPG between those grazing dryland or irrigated pasture in the negative binomial multivariable model. However, older calves and those that had not been dewormed prior to the study had statistically significantly lower egg counts. The Mediterranean climate in California may lead to the assumption that parasite burdens never reach critical levels because of the lack of precipitation during much of the dry season, which typically lasts from May through October. A number of factors play a role in the survival and movement of larvae in pastures including moisture, ambient temperature, and movement and survival in soil. Moisture on grass as well as warm ambient temperature have been shown to increase upward movement of trichostrongylid

Table 6. Coproculture results from fecal testing for herds undergoing fecal egg count reduction testing during a study on intestinal parasite burden and anthelmintic resistance in Northern California between March and August 2021

	Pretreatment						Posttreatment					
Ranch ID	No of larvae	%Cooperia	%Haemonchus	%Ostertagia	%Oesophagostomum	%Trichostrongylus	No of larvae	%Cooperia	%Haemonchus	%Ostertagia	%Oesophagostomum	%Trichostrongylus
D7	124	87	0	10	2	1	147	65	1	31	1	1
D8	104	92	0	7	1	0	148	96	0	0	4	0
D10	103	74	10	14	0	3	48	50	0	50	0	0
I1	146	93	0	7	0	0	171	83	0	17	0	0
I2	28	89	0	11	0	0	56	95	0	5	0	0
I3	131	76	0	24	0	0	120	62	0	38	0	0
I5	93	69	2	27	2	0	90	82	6	7	6	0
I6	101	94	6	0	0	0	120	75	0	23	2	0

larvae on grass blades (Silangwa and Todd, 1964) increasing the chances of being consumed through a grazing animal. Flooding, which is the most commonly used method of irrigation for irrigated pastures in California, is associated with vertical migration of larvae in the soil rather than migration on grass and may lead to lower infection rates of cattle than sprinkler irrigation (Uriarte and Gruner, 1994). *Ostertagia* larvae have been shown to be able to migrate through the soil and return to surface grass suggesting that soil may act as a reservoir for larvae (Krecek, 1988). *Cooperia oncophora* larvae were found to survive within soil during dry conditions in a laboratory setting and survival was more impacted by higher environmental temperatures (20 to 33 °C) compared to moderate temperatures (17 to 22.6 °C) than by drought conditions (Knapp-Lawitzke et al., 2016). Taken together, moisture alone does not seem to explain survival of larvae in pastures and grazing on dryland may not prevent infections even during the dry period. Age has been identified as a predictor for FECs with older animals shedding fewer eggs because of developed resistance to internal parasites (Charlier et al., 2020). In our study, we found there was significant variability in FECs between herds on either irrigated pasture or dryland, indicating that environmental conditions may not be the only factor responsible for intestinal parasite burden. Weather, climate, season, region, stocking rates and rotation, immunological, physiological, and health status of animals also play a role in the prevalence and degree of cattle nematodosis (Navarre, 2020). There is anecdotal evidence of fatal ostertagiasis despite a history of ivermectin treatment in a beef cow submitted for necropsy and diagnosed at the CAHFS laboratory (California Animal Health and Food Safety Laboratory, 2024). While cases of fatal parasitism may be rare under local environmental conditions, effective anthelmintic treatment may still be necessary to achieve a state of health and production for cattle in California.

Based on FECs, several herds in our sample likely had parasite burdens that may not merit anthelmintic treatment; however, some individuals clearly had reached the threshold where treatment was likely beneficial. There is no universally accepted threshold for treatment, but a geometric mean of >200 EPG has been determined as a cutoff above which parasitic gastroenteritis was observed in first-grazing season groups of calves in a meta-analysis of 85 studies (Shaw et al., 1998). Most herds in our study stayed well below this threshold. Other studies suggested an average (presumably arithmetic mean) fecal egg count of ≥100 EPG in a group as a threshold for treatment in grazing calves (Charlier et al., 2014). The use of FECs is unfortunately only poorly correlated with actual gastrointestinal parasite burden, however, due to its ease of use and low cost it is the method of choice for grazing calves (Charlier et al., 2023). Density dependence, i.e., competition leading to lower fecundity and increased worm mortality, as well as hypobiosis, i.e., arrested development of ingested L3 larvae during harsh environmental conditions partly explain the poor correlation between FECs and worm burden (Charlier et al., 2020). Fecundity as well as pathogenicity differs between species of nematodes (Amarante et al., 2014). In the present study, we found calves shedding between 0 and 1305 EPG with arithmetic mean EPG counts for groups between 6 and 322. While other genera were absent or rare, coproculture showed compositions of larvae that consisted between 55% and 98% of the genus *Cooperia,* with varying levels of *Ostertagia* (0% to 26.9%). The most important species in these genera in temperate climates are *Cooperia oncophora* and *Ostertagia ostertagi* (Charlier et al., 2020). *Cooperia* species reside in the duodenum and upper jejunum resulting in mucosal thickening, and subsequent reduction in weight gain (Charlier et al., 2020). *O. ostertagi* invade fundic glands in the abomasum, damaging the glandular tissue, leading to infiltration of the submucosa by inflammatory cells, and resulting in impaired abomasal function (Taylor et al., 1989; Fox, 1997; Mihi et al., 2013). As there was relative uniformity in the composition of nematode genera, with most herds having a majority of *Cooperia* spp. with some component of *Ostertagia* spp, we may assume that severity of parasite burden in terms of pathology was comparable among study herds.

Testing of fresh feces ahead of cattle processing would give producers a better understanding of whether anthelmintic treatment is necessary or not. However, one of the impediments to fecal testing for cattle is that very few laboratories offer the Mini FLOTAC test, which has the required accuracy to guide decisions on anthelmintic use. The McMasters assay offered more frequently has a resolution of 50 EPG, i.e one egg seen under the microscope translates to 50 EPG, while the Mini FLOTAC has a resolution of 5 EPG.

Noteworthy is also the fact that we did not find any samples that were positive for liver fluke eggs or lungworm larva. Fluke eggs rely on environmental moisture for survival as well as the intermediate snail host. We expected fluke eggs to be present in samples collected from calves on irrigated pasture and four of the participating herds reported the routine use of a flukicide, which is typically performed during late summer or fall when liver flukes have reached the adult stage (Howell and Williams, 2020). *Fasciola hepatica* has been described in cattle on the West coast (Pecoraro et al., 2022), but specific data on prevalence in California cattle is sparse to non-existent. According to the National Beef Quality Audit, 2016, 3.2% of livers from market cows and bulls nationwide were condemned at slaughter for human consumption due to the presence of liver flukes (National Cattlemen's Beef Association, 2016). Another source states that 24% of slaughtered cattle from 26 states in the United States are found to harbor either *F. hepatica* or *F. magna*, however no reference to that statement was cited (Howell and Williams, 2020). Regardless of actual prevalence, it is likely that infections are clustered within herds that are grazing where liver flukes occur. As the cattle in our study were young, flukes, if present, may not have had enough time to complete their life cycle and shed eggs in feces.

We also did not detect the presence of lungworm larvae in the tested samples. The Baermann test will yield inaccurate results >24 hours after defecation. We submitted all samples within 24 hours of collection to the laboratory conducting the testing but cannot guarantee that sample quality was optimal in all instances. Prevalence estimates of *Dictyocaulus viviparous* infections in cattle are equally sparse, but infections have been associated with cool, moist climates in the United States (Underwood et al., 2015). We included herds from the California north coast, with cooler temperatures and higher precipitation than in other regions that were sampled, and where conditions may have been more favorable for lungworm larvae. Despite inclusion of these higher risk herds, we were not able to detect any lungworm larvae in the submitted samples.

Fecal Egg Count Reduction Testing

None of the herds enrolled in the present study was able to achieve the desired FECR of ≥99% with a lower value of the CI of ≥90%. However, a number of factors may have influenced the results. We allowed ranchers to select the anthelmintic and the time of deworming of their choice to evaluate efficacy of current practices. None of the herds in the study measured the weight of cattle or used tools to estimate the weight, such as a heart girth tape. As the most common method of determining dosages by participants in the study was to use the same dose for all cattle, it is likely that incorrect dosing was common. We cannot tell, based on the available information, whether cattle were overdosed or underdosed. Based on experience of the researchers, often an "average" weight is chosen for dosing, but that average is often underestimated. In any case, inaccurate dosing will lead to unreliable FECRT results where efficacy is reduced because of underdosing rather than AR. It is also noteworthy that all participants selected an anthelmintic belonging to the class of ML. Only one of the herds (I4) listed a different drug class for the last dewormer used in the groups of cattle participating in the study, which was fenbendazole. As repeated use of the same class of anthelmintic, combined with underdosing and treatment of all cattle without allowing for refugia are often cited as reasons for AR development, the results of the current study come as no surprise. Two of the herds were treated with pour-on applications of dewormer, which may yield therapeutic failures due to poor drug absorption rather than AR. These therapeutic failures are attributed to allo- and self-licking, haircoat type and length, soiling of haircoat or poor application technique (Kaplan et al., 2023). We found several herds in the FECRT study arm that had very low FECs at enrollment that likely did not merit treatment at that time. WAAVP guidelines for sample sizes and follow-up intervals were updated after we had completed data collection. Samples sizes as required in the updated WAAVP guidelines could not be met with our study design, which may have also reduced the reliability of results.

In a recent study in young calves and their dams on California irrigated pasture, AR to either eprinomectin or doramectin products was observed in both calves and dams, although FECs in dams were quite low making estimates less reliable (Davy et al., 2023). Similarly, a study comparing weight gain and FECs in groups of steers originating from California and treated with either doramectin, eprinomectin, or a combination of doramectin and albendazole also found evidence of ML resistance while the combination treatment resulted in almost 100% efficacy in FECR (Edmonds et al., 2018). An alarming study from New Zealand reports the simultaneous resistance of *Cooperia* spp. and *Ostertagia* spp. to benzimidazole, ML and levamisole anthelmintics in cattle (Sauermann et al., 2024). The present study may add more evidence to the reality of AR to the ML treatments for cattle in the arid western United States.

Limitations of the current study include that we do not know whether animals were treated with the proper dose or whether anthelmintic drugs used had been properly stored prior to using in the FECRT, two conditions required by the WAAVP guidelines for FECRT. Further, the minimum number of eggs counted and animals included in testing to achieve the accuracy and precision outlined in the updated WAAVP guidelines were not always met (Kaplan et al., 2023). Given that most FECRT results were well below the threshold for susceptibility, they are, however, highly indicative of the presence of AR in these herds.

Despite the limitations, the study provided cross-sectional data on current intestinal parasite burdens including prevailing genera in growing cattle grazed on both irrigated and dryland pasture as well as usage patterns for anthelmintics by ranchers. Additional data on FECRT as outlined by the 2023 WAAVP would be desirable.

Additionally, regardless of whether AR is present or not, current practices for deworming growing grazing beef cattle in California are not achieving their goal. Efficacy of ML drugs as they are currently used by cattle producers is reduced as demonstrated in the FECRTs conducted in this study. Furthermore, not all herds that were treated had evidence of intestinal parasite infections based on laboratory testing.

A paradigm shift in anthelmintic treatment may be required to be able to continue the successful use of these drugs. Potential avenues forward could include testing before treatment, more accurate dosing of drugs, refugia, or combination treatments. Changing habits is difficult, especially if it requires extra steps that may be seen as an additional cost. However, the current practices do not seem to be cost-effective since treatment is either ineffective or not necessary.

CONCLUSIONS

Nematode parasite loads in northern California growing grazing cattle are variable with some herds showing FECs that may not merit treatment. No liver fluke eggs or lungworm larvae were detected on fecal testing in the study population. FECRT showed widespread resistance to ML type dewormers in the tested herds, although results have to be interpreted with caution. A different approach to anthelmintic treatment in grazing cattle may be necessary to avoid rendering ML class drugs completely ineffective.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

Author Contributions

Gabriele Maier (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—original draft), Phillip Torcal (Investigation, Writing—original draft), Jeffery Stackhouse (Investigation, Writing—review & editing), Laura Snell (Investigation, Writing—review & editing), Grace Woodmansee (Investigation, Writing—review & editing), Josh Davy (Investigation, Writing—review & editing), and Larry Forero (Investigation, Writing—review & editing)

Literature Cited

Amarante, M. R., C. C. Bassetto, J. H. Neves, and A. F. Amarante. 2014. Species-specific PCR for the identification of Cooperia curticei (Nematoda: Trichostrongylidae) in sheep. J. Helminthol. 88:447–452. doi:10.1017/S0022149X13000412

California Animal Health and Food Safety Laboratory. 2024. Bovine. CAHFS Connection newsletter. https://cahfs.vetmed.ucdavis.edu/sites/g/files/dgvnsk2461/files/media/documents/CAHFS_Connection_2024_03.pdf

California Department of Fish and Wildlife (CDFW). 2021. Atlas of the biodiversity of California. 2nd ed. Sacramento (CA): State of California.

Charlier, J., J. Hoglund, E. R. Morgan, P. Geldhof, J. Vercruysse, and E. Claerebout. 2020. Biology and epidemiology of gastrointestinal nematodes in cattle. Vet. Clin. North Am. Food Anim. Pract. 36:1–15. doi:10.1016/j.cvfa.2019.11.001

Charlier, J., E. R. Morgan, L. Rinaldi, J. van Dijk, J. Demeler, J. Hoglund, H. Hertzberg, B. Van Ranst, G. Hendrickx, J. Vercruysse, et al. 2014. Practices to optimise gastrointestinal nematode control on sheep, goat and cattle farms in Europe using targeted (selective) treatments. Vet. Rec. 175:250–255. doi:10.1136/vr.102512

Charlier, J., D. J. Williams, N. Ravinet, and E. Claerebout. 2023. To treat or not to treat: diagnostic thresholds in subclinical helminth infections of cattle. Trends Parasitol. 39:139–151. doi:10.1016/j.pt.2022.11.014

Ciordia, H., H. C. McCampbell, G. V. Calvert, and R. E. Plue. 1984. Effect of ivermectin on performance of beef cattle on Georgia pastures. Am. J. Vet. Res. 45:2455–2457.

Claerebout, E., and J. Vercruysse. 2000. The immune response and the evaluation of acquired immunity against gastrointestinal nematodes in cattle: a review. Parasitology 120:S25–S42. doi:10.1017/s0031182099005776

Coles, G. C., C. Bauer, F. H. M. Borgsteede, S. Geerts, T. R. Klei, M. A. Taylor, and P. J. Waller. 1992. World association for the advancement of veterinary parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44:35–44. doi:10.1016/0304-4017(92)90141-u

Cringoli, G., M. P. Maurelli, B. Levecke, A. Bosco, J. Vercruysse, J. Utzinger, and L. Rinaldi. 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat. Protoc. 12:1723–1732. doi:10.1038/nprot.2017.067

Davy, J. S., L. C. Forero, S. Strickler, J. Gillespie, and G. U. Maier. 2023. Comparison of deworming strategies for pre-weaned beef calves. Vet. Parasitol. 322:110005. doi:10.1016/j.vetpar.2023.110005

Edmonds, M. D., A. F. Vatta, A. A. Marchiondo, H. B. Vanimisetti, and J. D. Edmonds. 2018. Concurrent treatment with a macrocyclic lactone and benzimidazole provides season long performance advantages in grazing cattle harboring macrocyclic lactone resistant nematodes. Vet. Parasitol. 252:157–162. doi:10.1016/j.vetpar.2018.02.009

Fox, M. T. 1997. Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. Vet. Parasitol. 72:285–97; discussion 297. doi:10.1016/s0304-4017(97)00102-7

Gasbarre, L. C. 2014. Anthelmintic resistance in cattle nematodes in the US. Vet. Parasitol. 204:3–11. doi:10.1016/j.vetpar.2014.03.017

Geurden, T., C. Chartier, J. Fanke, A. F. di Regalbono, D. Traversa, G. von Samson-Himmelstjerna, J. Demeler, H. B. Vanimisetti, D. J. Bartram, and M. J. Denwood. 2015. Anthelmintic resistance to ivermectin and moxidectin in gastrointestinal nematodes of cattle in Europe. Int. J. Parasitol. Drugs Drug Resist. 5:163–171. doi:10.1016/j.ijpddr.2015.08.001

Howell, A. K., and D. J. L. Williams. 2020. The epidemiology and control of liver flukes in cattle and sheep. Vet. Clin. North Am. Food Anim. Pract. 36:109–123. doi:10.1016/j.cvfa.2019.12.002

Kaplan, R. M., M. J. Denwood, M. K. Nielsen, S. M. Thamsborg, P. R. Torgerson, J. S. Gilleard, R. J. Dobson, J. Vercruysse, and B. Levecke. 2023. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses and swine. Vet. Parasitol. 318:109936. doi:10.1016/j.vetpar.2023.109936

Kelleher, A. C., B. Good, T. de Waal, and O. M. Keane. 2020. Anthelmintic resistance among gastrointestinal nematodes of cattle on dairy calf to beef farms in Ireland. Ir. Vet. J. 73:12. doi:10.1186/s13620-020-00167-x

Knapp-Lawitzke, F., G. von Samson-Himmelstjerna, and J. Demeler. 2016. Elevated temperatures and long drought periods have a negative impact on survival and fitness of strongylid third stage larvae. Int. J. Parasitol. 46:229–237. doi:10.1016/j.ijpara.2015.10.006

Krecek, R. C., and K. D. Murrell. 1988. Observations on the ability of larval ostertagia ostertagi to migrate through pasture soil. Proc. Helminthol. Soc. Wash. 55:24–27.

Macon, D. K., S. Barry, T. Becchetti, J. S. Davy, M. P. Doran, J. A. Finzel, H. George, J. M. Harper, L. Huntsinger, R. S. Ingram, et al. 2016. Coping with drought on California rangelands. Rangelands 38:222–228. doi: 10.1016/j.rala.2016.06.005

Maurizio, A., A. F. di Regalbono, and R. Cassini. 2021. Quantitative monitoring of selected groups of parasites in domestic ruminants: a comparative review. Pathogens 10:1173. doi:10.3390/pathogens10091173

Mihi, B., F. Van Meulder, M. Rinaldi, S. Van Coppernolle, K. Chiers, W. Van den Broeck, B. Goddeeris, J. Vercruysse, E. Claerebout, and P. Geldhof. 2013. Analysis of cell hyperplasia and parietal cell dysfunction induced by Ostertagia ostertagi infection. Vet. Res. 44:121. doi:10.1186/1297-9716-44-121

National Cattlemen's Beef Association. 2016. National beef quality assurance quality audit market cow and bull executive summaryCentennial (CO): Cattlemen's Beef Board and National Cattlemen's Beef Association.

Navarre, C. B. 2020. Epidemiology and control of gastrointestinal nematodes of cattle in southern climates. Vet. Clin. North Am. Food Anim. Pract. 36:45–57. doi:10.1016/j.cvfa.2019.11.006

Pecoraro, H. L., B. L. S. Stenger, L. E. Rice, and B. T. Webb. 2022. Gross and histologic description of trematodosis in fetal and neonatal beef calves in North Dakota and Minnesota. J. Vet. Diagn. Invest. 34:870–873. doi:10.1177/10406387221109619

Sabatini, G. A., F. de Almeida Borges, E. Claerebout, L. S. Gianechini, J. Höglund, R. M. Kaplan, W. D. Zanetti Lopes, S. Mitchell, L. Rinaldi, G. von Samson-Himmelstjerna, et al. 2023. Practical guide to the diagnostics of ruminant gastrointestinal nematodes, liver fluke and lungworm infection: interpretation and usability of results. Parasit. Vectors 16:58. doi:10.1186/s13071-023-05680-w

Sauermann, C., T. Waghorn, C. Miller, and D. Leathwick. 2024. Simultaneous resistance to multiple anthelmintic classes in nematode parasites of cattle in New Zealand. Vet. Parasitol. 325:110079. doi:10.1016/j.vetpar.2023.110079

Shaw, D. J., J. Vercruysse, E. Claerebout, and P. Dorny. 1998. Gastrointestinal nematode infections of first-grazing season calves in Western Europe: associations between parasitological, physiological and physical factors. Vet. Parasitol. 75:133–151. doi:10.1016/s0304-4017(97)00213-6

Silangwa, S. M., and A. C. Todd. 1964. Vertical migration of trichostrongylid larvae on grasses. J. Parasitol. 50:278–285.

Stuedemann, J. A., H. Ciordia, G. H. Myers, and H. C. McCampbell. 1989. Effect of a single strategically timed dose of fenbendazole on cow and calf performance. Vet. Parasitol. 34:77–86. doi:10.1016/0304-4017(89)90167-2

Taylor, L. M., J. J. Parkins, J. Armour, P. H. Holmes, K. Bairden, A. M. Ibarra-Silva, S. K. Salman, and P. N. McWilliam. 1989. Pathophysiological and parasitological studies on Ostertagia ostertagi infections in calves. Res. Vet. Sci. 46:218–225.

Torgerson, P. R., M. Paul, and R. Furrer. 2014. Evaluating faecal egg count reduction using a specifically designed package “eggCounts” in R and a user friendly web interface. Int. J. Parasitol. 44:299–303. doi:10.1016/j.ijpara.2014.01.005

Underwood, W. J., R. Blauwiekel, M. L. Delano, R. Gillesby, S. A. Mischler, and A. Schoell. 2015. Biology and diseases of ruminants (Sheep, Goats, and Cattle). In: Fox, J. G., G. M. Otto, M. T. Whary, L. C. Anderson, and K. R. Pritchett-Corning, editors. Laboratory animal medicine. San Diego (CA): Elsevier. doi:10.1016/B978-0-12-409527-4.00015-8

United States Agricultural Statistics Service. 2022. Cattle and Calves - Inventory: 2022 and 2017. Ithaca (NY): United States Department of Agriculture.

Uriarte, J., and L. Gruner. 1994. Development and distribution of bovine trichostrongyle infective larvae on a pasture irrigated by flooding or by spraying. Parasitol. Res. 80:657–663. doi:10.1007/BF00932949

Wang, C., P. R. Torgerson, R. M. Kaplan, M. M. George, and R. Furrer. 2018. Modelling anthelmintic resistance by extending eggCounts package to allow individual efficacy. Int. J. Parasitol. Drugs Drug Resist. 8:386–393. doi:10.1016/j.ijpddr.2018.07.003

Wiggin C. J. and Gibbs H. C. 1989. Studies of the immunomodulatory effects of low-level infection with Ostertagia ostertagi in calves. Am. J. Vet. Res. 50:1764–1770.

Wohlgemuth, K, J. J. Melancon, H. Hughes, and M. Biondini 1988. Relationships between weaning weights of North Dakota beef calves and treatment of their dams with ivermectin. Agri-Practice 9:23–26.