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## Abstract

During the United States Department of Agriculture (USDA) National Animal Health Monitoring System's (NAHMS) 2007–2008 beef study, producers from 24 states were offered the opportunity to evaluate their animals for internal parasites and for overall responses to treatment with anthelmintics. A lapse of 45 d was required between initial sampling and any previous treatments. Choice of anthelmintic (oral benzimidazoles, and both injectable and pour-on endectocides) was at the discretion of the producer so as not to alter the local control programs. Fresh fecal samples were collected from 20 animals, or from the entire group if less than 20, then randomly assigned to 1 of 3 participating laboratories for examination. Analyses consisted of double centrifugation flotation followed by enumeration of strongyle, *Nematodirus*, and *Trichuris* eggs (the presence of coccidian oocysts and tapeworm eggs was also noted). Where strongyle eggs per gram (epg) exceeded 30, aliquots from 2 to 6 animals were pooled for egg isolation and polymerase chain reaction (PCR) analysis for the presence of *Ostertagia*, *Cooperia*, *Haemonchus*, *Oesophagostomum*, and *Trichostrongylus*. Results from 72 producers (19 States) indicated that fecal egg count reductions were $< 90\%$ in 1/3 of the operations. All operations exhibiting less than a 90% reduction had used pour-on macrocyclic lactones as the anthelmintic treatment. While some of these less than expected reductions could have been the result of improper drug application, PCR analyses of the parasite populations surviving treatment, coupled with follow-up studies at a limited number of sites, indicated that less than expected reductions were most likely due to anthelmintic resistance in *Cooperia* spp. and possibly *Haemonchus* spp.

## Résumé

*Pendant l'étude de 2007–2008 chez les bovins effectuée par le Système national de surveillance des maladies animales (NAHMS) du Département de l'agriculture des États-Unis (USDA), des producteurs provenant de 24 états américains se sont vus offrir l'opportunité de faire évaluer leurs animaux pour la présence de parasites internes et pour leur réponse globale à un traitement avec des anthelminthiques. Un délai de 45 j était requis entre l'échantillonnage initial et un traitement antérieur. Le choix d'un anthelminthique (benzimidazole oral, et des endectocides injectables et en solution à verser) était à la discrétion du producteur afin de ne pas altérer les programmes de contrôle locaux. Des échantillons de fèces fraiches ont été prélevés de 20 animaux, ou de tout le groupe si moins de 20, puis ils ont été acheminés de manière aléatoire à un des trois laboratoires participants pour fin d'examen. L'analyse consistait en une double centrifugation par flottaison suivie d'une énumération des strongles, de* Nematodirus, *et d'œufs de* Trichuris *(la présence d'ookystes de coccidie et d'œufs de vers plats fut également notée). Lorsque le nombre d'œufs de strongles par gramme dépassait 30, des aliquots de 2 à 6 animaux étaient regroupés pour isolement des œufs et et soumis à une réaction d'amplification en chaine par la polymérase (PCR) pour détecter la présence d'*Ostertagia, *de* Cooperia, *d'*Haemonchus, *d'*Oesophagostomum, *et de* Trichostrongylus. *Les résultats provenant de 72 producteurs (19 états) indiquent que les réductions dans le dénombrement des œufs dans les fèces étaient de $< 90$ % dans le tiers des opérations. Toutes les opérations montrant une réduction de moins de 90 % avaient utilisé des lactones macrocycliques en solution à verser comme traitement anthelminthique. Alors que certaines de ces réductions moindres que prévues puissent être le résultat d'une mauvaise application du produit, les analyses par PCR des populations de parasites survivantes au traitement, combinées aux études de suivis à un nombre limité de sites, indiquent que les réductions moindres que prévues étaient fort probablement dues à la résistance aux anthelminthiques chez* Cooperia *spp. et possiblement* Haemonchus *spp.*

*(Traduit par Docteur Serge Messier)*

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# Introduction

Development of the anthelmintics currently used has resulted in important changes in how cattle are raised in the United States (US). The high efficacy against a broad spectrum of parasite genera combined with large margins of safety changed the timing, manner, and frequency of drug applications. Prior to the discovery and development of the macrocyclic lactone and benzimidazole classes of anthelmintics, drugs were used to therapeutically treat parasite-compromised animals. The development of safer drugs and application systems that minimized animal handling led to strategically timed deworming. These programs have attempted to deliver drugs during critical time points in parasite transmission cycles to maximize the numbers of parasites exposed to the drugs (1). The goal has been to attain optimal animal productivity through the reduction of parasite transmission by reducing the number of infective larvae on pasture. As a result of these new and highly efficient control programs, producers have been able to employ higher stocking rates and, in many cases, have reduced or eliminated non-chemical adjuncts in their parasite control programs (2).

Anthelmintic-based control programs have been extremely profitable for many cattle raisers in the US. At the same time, data from small ruminant production systems globally have raised the concern that programs that rely strictly upon drug administration without regard for good pasture parasite management will lead to more rapid selection of drug resistance in nematode populations (3). Such selection has been well documented in small ruminant species in which gastrointestinal nematodes resistant to all classes of anthelmintics have been identified (4). In cattle systems, there have been documented reports of drug resistance to ivermectin and benzimidazoles by *Cooperia* spp. in New Zealand (5–7), to ivermectin by *Cooperia* spp. in Great Britain (8), to both macrocyclic lactones and benzimidazoles by *Haemonchus* spp., and to the macrocyclic lactones by *Cooperia* spp. in Argentina (9). The first documented case of resistance within the US to the avermectins/milbemycins involved stocker cattle raised under an intensive grazing system in the upper midwestern US in the summer and fall of 2004 (10,11). This was quickly followed by a second report from the western US (12).

Recently, there has been an increasing perception that resistance to the most commonly used cattle anthelmintics has been on the rise in the US. In an attempt to gain insights into the effectiveness of producer applied anthelmintic treatments, as part of the National Animal Health Monitoring System (NAHMS) 2007–2008 beef study of cow-calf operations, producers were given the opportunity to assess the effect of their current anthelmintic program on reducing fecal egg counts (FEC) in weaned calves on pasture.

# Materials and methods

## Study locations and sample collection

Beef cow-calf operations that agreed to participate in a national study of animal health and management conducted by the United States Department of Agriculture (USDA) NAHMS were eligible to submit samples for evaluation for intestinal parasite eggs. This population of operations was described previously (13,14). Operations that collected fecal samples to evaluate egg counts and planned to treat the calves with an anthelmintic were eligible for a second sample collection approximately 14 d after treatment.

Producers were given instructions and all materials necessary to participate in the study, including shipping supplies (paid for by the NAHMS program). Sample collection took place between March 1 and December 2, 2008 at the producer's discretion, from weaned calves (6 to 18 mo of age) that had been grazing at least 4 wk and had not been treated with anthelmintics in the previous 45 d. Using new gloves with each sample, producers collected fresh feces from no more than 20 calves either directly from the rectum or from an observed drop. Each sample, 3 to 4 cm in diameter ("the size of a golf ball") was transferred to individual plastic bags, chilled overnight, and then shipped with ice packs to one of 3 randomly assigned laboratories.

For producers that planned to treat their calves with an anthelmintic, the first set of samples were collected randomly at the time of treatment or immediately prior to treatment; a second set of samples was again randomly collected approximately 14 to 16 d after the anthelmintic treatment. During both collections, samples were taken randomly from the same group of animals. Both sets of samples were submitted to the same designated laboratory [Colorado State University; USDA, Agricultural Research Service (ARS), Beltsville; University of Minnesota].

At the time of sample collection, producers were asked to complete a short questionnaire requesting information on the number of animals in the group sampled, last treatment with an anthelmintic, and product used. Data on routine deworming practices were available from a previously administered questionnaire (Ballweber et al, 2008, Colorado State University, personal communication).

## Laboratory procedures for generating fecal egg counts (FEC)

Upon receipt, fecal samples were given reference numbers then refrigerated until analyzed. Eggs were quantified using the Wisconsin Double Centrifugal Flotation or the Modified Wisconsin techniques (15,16) according to procedures routinely used in that laboratory. Eggs were morphologically identified as strongyle, *Nematodirus*, or *Trichuris*. All samples were further evaluated for the presence of coccidia oocysts and *Moniezia* eggs.

Pre-treatment egg counts (Phase 1) were compared to post-treatment counts (Phase 2) and expressed as a percent FEC. Fecal egg count reduction (FECR) was calculated for each operation as follows:

$$\text{FECR} = [(\text{Avg FEC Pre-treatment}) - (\text{Avg FEC Post-treatment})]/(\text{Avg FEC Pre-treatment}) \times 100$$

where Avg is average. The industry standard of $< 90\%$ reduction (17,18) following anthelmintic treatment was used to define the presence of drug resistance in a parasite population.

## Egg DNA isolation and polymerase chain reaction (PCR)

For genetic analysis, eggs were purified by zinc sulfate flotation (19), washed extensively in tap water, and then frozen at −80°C in 0.5 mL PCR tubes prior to shipment to the ARS laboratory in

**Table I. Location and results of operations submitting acceptable samples for fecal egg counts (FEC)**

| Region | State | Number of operations submitting sample | Number of FECRT ≤ 90% | Percentage of operations ≤ 90% by region |
|---|---|---|---|---|
| Southeast | Alabama | 1 | 1 | 33% |
| | Louisiana | 3 | 0 | |
| | Oklahoma | 4 | 2 | |
| | Tennessee | 1 | 0 | |
| | Texas | 1 | 1 | |
| | Virginia | 2 | 0 | |
| Central | Iowa | 6 | 5 | 58% |
| | Kansas | 7 | 6 | |
| | Missouri | 4 | 2 | |
| | Nebraska | 6 | 1 | |
| | North Dakota | 8 | 4 | |
| West | California | 1 | 1 | 28% |
| | Colorado | 2 | 1 | |
| | Idaho | 4 | 0 | |
| | New Mexico | 1 | 0 | |
| | Oregon | 4 | 0 | |
| | Wyoming | 6 | 3 | |

Beltsville, Maryland, for PCR analysis. A minimum of 100 eggs was required to maximize representation of all infecting species in the sample. Upon arrival, the samples were processed for DNA and PCR amplification as described (14) using a non-multiplex format and genus specific primer pairs (20) for *Cooperia*, *Ostertagia*, *Haemonchus*, *Oesophagostomum*, and *Trichostrongylus*. The PCR products were analyzed on a 2% NuSieve® 3:1 agarose gel (Lonza Rockland; Rockland, Maine, USA) subsequently stained with ethidium bromide. The PCR fragments migrating at 151 bp (*Cooperia* spp.), 257 bp, (*Ostertagia* spp.), 176 bp (*Haemonchus* spp.), 329 bp (*Oesophagostomum* spp.), and 243 bp (*Trichostrongylus* spp.) were scored for the presence of the respective parasites.

## Follow-up studies

Results of FEC were returned to the participating producers. These results included an interpretation of the results by the laboratory performing the analyses. Three participants submitted additional fecal samples as a follow-up to the NAHMS study. The follow-up samples were taken from subsets of 20 individual animals randomly chosen from the same group used in the Phase 1 and Phase 2 samplings. Producers collected fecal samples directly from the rectum of 20 animals and then retreated the animals with the same drug used for the earlier treatment. Two of these 3 participants also expressed interest in testing a different class of anthelmintic in their animals. To accommodate these requests, additional groups of 20 animals were identified from the originally sampled animals. If a pour-on was used, animals receiving the pour-on were physically isolated from animals receiving non-pour-on treatment. Fourteen days after drug application, the same 20 animals per group were sampled a second time by collection of feces from the rectum. All samples were sent to the same laboratory doing the initial evaluation and handled as described previously for FEC.

## Statistical analyses

Analyses of FEC were done using computer software (SAS/STAT, Version 9.2; SAS Institute, Cary, North Carolina, USA) (21). The percent reduction in strongyle counts (between Phase 1 and Phase 2) by treatment grouping was calculated for each farm/phase based upon the mean strongyle count across the 20 samples. For each farm, the difference between the Phase 1 mean and the Phase 2 mean was calculated and classified into 1 of 2 categories: $< 90\%$ reduction or $\geq 90\%$ reduction in FEC. A Fisher's exact test was used to test for associations between treatment grouping and percent reduction category. The statistical method used in this study had been extensively evaluated and verified elsewhere (22) for reliability and reproducibility in analyzing FEC. An extension of the Box–Cox transformation accounted for normal variations wherein both raw FEC and log(FEC + 1) values reduced skewness and kurtosis, and approached FEC normality. A minimum of 17 animals were required to ensure that sampling accounted for all intervals of highly skewed distributions (23).

# Results

## Results and participation in Phase 2 studies

A total of 72 operations submitted both the pre-treatment and post-treatment samples. Of these, a total of 61 met all criteria of sample submission and were used in further analyses. Table I indicates the locations of operations submitting a Phase 2 sample and

**Table II. Effect of anthelmintic treatment on fecal egg counts (FEC). Results presented as both percentage and (total number) of operations**

| Reduction | All operations | Operations where pre-treatment, FEC ≥ 10 | Operations where pre-treatment, FEC ≥ 20 |
|---|---|---|---|
| ≥ 90% | 55.7 (34) | 64.8 (35) | 66.7 (32) |
| < 90% | 44.3 (27) | 35.2 (19) | 33.3 (16) |

**Table III. Mean fecal egg counts (FEC) before and after drug treatment by geographic region**

| Geographic region | FECRT | Number of operations | Mean EPG before treatment | Mean EPG after treatment | Mean FECRT |
|---|---|---|---|---|---|
| South | ≤ 90 | 4 | 32.3 | 8.8 | 73% |
| | ≥ 90 | 7 | 103.1 | 4 | 96% |
| Central | ≤ 90 | 18 | 30.8 | 16.4 | 47% |
| | ≥ 90 | 14 | 33.9 | 0.7 | 98% |
| West | ≤ 90 | 5 | 19.2 | 20.4 | 0% |
| | ≥ 90 | 13 | 13.3 | 0.6 | 95% |
| All operations | ≤ 90 | 27 | 29.5 | 15.4 | 48% |
| | ≥ 90 | 34 | 34 | 1.4 | 96% |

EPG — eggs per gram.

**Table IV. Number and percentage of operations not reaching ≥ 90% reduction in fecal egg counts (FEC) by drug formulation**

| Drug formulation | Number ≤ 90% | Number ≥ 90% | Percentage ≤ 90% |
|---|---|---|---|
| Brand name pour-on ML | 13 | 14 | 48% |
| Generic pour-on ML | 12 | 4 | 75% |
| Brand name injectable ML | 2 | 10 | 17% |
| Generic injectable ML | 0 | 1 | 0% |
| Oral BZ | 0 | 5 | 0% |

ML — macrocyclic lactone; BZ — benzimidazoles.

the results of the Fecal Egg Count Reduction Test (FECRT). Greater than 1/3 of the operations tested did not meet the 90% FECRT cut-off value (Tables I and II). To ensure that these results were not overly influenced by samples with very low FEC values, the analyses were repeated excluding pre-treatment means of < 10 eggs per gram (epg) and again after excluding operations with values < 20 epg. The exclusion of these samples had only a slight effect on the percentage not reaching the 90% FECRT cut-off in those samples with a pre-treatment mean of < 10 epg (Table II). Failure to reach the 90% cut-off was not geographically restricted (Table III). Pre-treatment egg counts for operations with < 90% reductions were similar in number to operations with ≥ 90% reductions with the exception of the South (Table III). In general, the average FECRT value where efficacy was ≥ 90% was approximately 95%, while the average for operations < 90% was approximately 50% (Table III). In all cases, operations that failed to reach the 90% cut-off used either a generic or brand name macrocyclic lactone, and included both pour-on and injectable formulations (Table IV).

## Results of PCR analyses

To identify the parasites surviving after anthelmintic treatment, PCR analysis was done on eggs recovered from the post-treatment samples. Eighteen pre- and post-treatment pairs yielded DNA that was of sufficient quality for further analyses. The dominant parasite eggs found after treatment were *Cooperia* spp. (95%). This percentage was relatively unchanged from that observed in pre-treatment samples, i.e., 88% (Table V). The percentage of samples containing *Haemonchus* eggs was similar in pre-treatment (56%) and post-treatment (50%) samples, albeit the overall frequency was roughly half that observed for *Cooperia.* Other parasite genera (*Ostertagia, Oesophagostomum,* and *Trichostrongylus*) were substantially reduced in the post-treatment samples relative to the pre-treatment samples (Table V). In general, anthelmintic treatment reduced the parasite genera in the feces in roughly two-thirds of the operations (12 of 18) where PCR was done on both pre- and post-treatment samples (Table VI).

## Results of follow-up studies

A total of 3 operations participated in follow-up studies. Two producers were concerned that the samples they submitted exhibited FECR of 51% and 59%. The third participant was concerned that the mean FEC for the pre-treatment samples was high (265 epg) indicating that current parasite control procedures were less than optimal. Upon retesting the previously used treatment protocol, 2 of

**Table V. Samples containing indicated genera of strongyle nematodes as determined by polymerase chain reaction (PCR) analysis of DNA derived from fecal eggs**

| Parasite genus | Pre-treatment sample | Post-treatment samples |
|---|---|---|
| *Cooperia* | 88% | 95% |
| *Ostertagia* | 79% | 40% |
| *Haemonchus* | 56% | 50% |
| *Oesophagostomum* | 38% | 5% |
| *Trichostrongylus* | 3% | 0 |

the 3 operations exhibited FECRT values below 90% (i.e., 24% and 73%) whereas the third operation demonstrated a reduction of 93%.

# Discussion

Over the last decade, there has been an increase in the number of anecdotal reports by cattle producers indicating disappointment in production results following anthelmintic treatment. This has led to speculation that cattle parasites have begun to demonstrate resistance to the most widely used anthelmintics. Anthelmintic resistance is common worldwide in nematode parasites of small ruminants (4), and has been reported in cattle in New Zealand and Great Britain (5,6,8). In a New Zealand study, encompassing a comparable number of farms ($n$ = 62) as the present study, Waghorn, et al (7) found that resistance to ivermectin was evident on 92% of the farms tested. The first documented occurrence of such resistance in the US was seen in a stocker operation in the upper Midwest (10,11). These reports were initially viewed as defining a unique incident arising from an intensively managed operation rather than an indication that drug resistance was becoming a concern for successful parasite control in US cattle operations. The study presented herein was undertaken to address this dichotomy and to assess the level of anthelmintic resistance in US cattle operations.

This first challenge in such an undertaking was to develop a reasonably accurate, precise, and affordable means to detect anthelmintic resistance. At present, the only method to determine the actual number and species of GI nematodes in a host is to necropsy the animal and recover, enumerate, and identify the parasites in the digestive tract (24). Even this exacting methodology requires a fairly large number of animals due to the skewed distribution of these parasites in their host. It also suffers from a number of logistical problems including the need to handle the tissues soon after death of the animal, and recovering and treating the large volumes of biological effluent resulting from thoroughly washing the digestive tract. As such, this method of testing is impractical for a survey of large numbers of cattle operations. Because of these limitations, the standard technique to estimate GI nematode numbers in a host has been the counting of parasite eggs in the feces.

Historically FEC have been used as the method of choice because of the relative ease in acquiring the sample and the non-invasive nature of the test. The standard means to assess drug efficacy without slaughter of the host has been the FECRT. This test requires sampling the animal at the time of treatment and again at some time after treatment. This methodology has been endorsed by major

**Table VI. Operations where the number of parasite genera present in the post-treatment sample was reduced after anthelmintic treatment as determined by polymerase chain reaction (PCR)**

| Number of operations | Operations in which genera were unchanged | Operations in which genera were reduced |
|---|---|---|
| 18 | 6 | 12 |

veterinary parasitology groups, and standard procedures have been developed (25). In most cases, the requirements have called for sampling 5 to 10 animals, and to sample the same animals before and after treatment to reduce variance in the assay (25).

In planning this study, it became evident that the requirement to sample the same animals before and after treatment would demonstrably reduce voluntary producer participation that is a hallmark of NAHMS surveys and, as such, impair the overall study. A previous report (23) provided some analyses of the precision of this methodology (not obtaining samples from the same animals) and the key sources of variation. Important information gleaned from this study included: i) the repeatability of the procedure is approximately 0.7 and that repeated sampling of the animals reduces the variance, ii) these reductions in variance are small and that after 3 samplings the variance reduction is minimal, and iii) the over-dispersed nature of FEC within a group of animals requires a within group size of at least 17 to account for all intervals in the distribution (if the group contained $< 17$ animals then all animals are sampled) (23).

Using data from that study to gain additional information about the variances associated with FEC, a second study was performed (22) that parsed the variances associated with the counting procedure. The study concluded that the 2 largest sources of variation were between different calves and between samples taken on consecutive days from the same calf. These 2 variables accounted for 80% to 85% of the total variance in FEC. From this we concluded that FEC are a reliable measure of the group, but are less reliable when assessing an individual animal without repeated sampling. This validated our approach to garner increased participation of producers by not requiring repeat sampling of the same animals. Based on these analyses, we used the FECRT to measure the change in a group mean rather than an individual animal, and required that 20 instead of 17 (23) different individuals be sampled 14 to 16 d after drug treatment. This number for group size is in agreement with that derived in a recent paper by Levecke, et al (18). Although sampling the same animals each time might affect the variance, this would have knowingly reduced the total number of participating operations and thus reduce substantially our ability to infer broader implications from the final data set.

Other factors that were considered in defining the sampling protocol were the time between treatment and sampling, the counting technique, and whether or not there should be a threshold egg count for inclusion in the study. With respect to time between treatment and second sampling (Phase 1 and Phase 2), samples taken $< 14$ d or $> 16$ d after treatment were excluded to ensure that the drug was given sufficient time to act, while providing insufficient time for reinfection and subsequent parasite patency. Regarding

the counting methodology, it was determined that a modified Wisconsin flotation was the only effective procedure. As practiced in the participating laboratories, this methodology had sensitivity between 0.5 and 3 epg. In contrast, the minimal detectable limit of the McMaster technique is 25, 50, or 100 epg depending upon the version used. The use of this methodology on cattle samples that generally have averages $< 100$ epg was deemed inappropriate in light of research suggesting that this technique cannot be used on samples where the egg counts are $< 100$ epg (26). Given the greater sensitivity of the modified Wisconsin technique, we used all data collected, and then examined the effect of removing the low mean epg operations. As seen in Table I removal of the low epg operations had very little effect on the percentages of operations demonstrating $< 90\%$ FECRT values. We ascribe this in large part to the sensitivity of the technique. Determination of a threshold epg value will require much more analysis than is provided in this study, but it is plain that such a threshold would define the sensitivity of the detection methods used. In the present study, the results were not substantially influenced by means of $< 10$ epg. Arithmetic means were used in all analyses based on the observation of Dobson, et al (27).

In over 1/3 of the operations tested, anthelmintic treatment resulted in $< 90\%$ reduction in FEC approximately 2 wk after treatment. The FECRT data are key components of licensing new anthelmintics where the average FECRT value for all trials submitted must be $\geq 90\%$. It is clear that the efficiencies of treating with macrocyclic lactones today in US commercial operations are not the same as the efficacies generated when the drugs were first licensed. Additionally, all operations in which the 90% levels were not reached had used either a generic or brand name macrocyclic lactone in either a pour-on or injectable formulation. The vast majority of the treatment failures that did not reach 90% were seen when pour-on formulations were used. Because the use of a pour-on macrocyclic lactone is by far the most common form of nematode control, a high percentage of current nematode control programs are not meeting the expectations in cow-calf operations. Examination of the PCR data indicated that the lack of efficacy is biased toward members of the genus *Cooperia,* and possibly *Haemonchus*. This is consistent with results found in the first demonstrated case of macrocyclic lactone resistance in the US (10,11).

There are 3 possible explanations why the expected levels of the FECRT were not reached. The first involves consistent underdosing resulting from errors in weight estimation of the animals when macrocyclic lactones were used. While this could explain some of the failures, it is unlikely that nearly half of the operations were from underdosing. Nonetheless, if underdosing is the cause, more effort must be put forth to educate producers as to the dangers of inadvertent underdosing when pour-on macrocyclic lactones are used for parasite control. A second and obvious explanation is that some animals were simply missed during anthelmintic treatment. Again this may explain some of the data; however, PCR results argue against this because the nematode populations changed in most operations after anthelmintic treatment, i.e., fewer genera present, where reductions were observed in all genera except *Cooperia* and *Haemonchus*. This would not have occurred in missed animals. The third and most telling explanation of these data is that resistance to the macrocyclic lactones has unilaterally appeared throughout the US in some genera of cattle GI nematodes. This increase in resistance is substantially manifested where pour-on formulations are used. Recent published reports indicate that adsorption of pour-ons is highly variable and is influenced by animal behavior (28). As such, resistance or resilience towards the drugs would be expected to appear first where this mode of application is being used. It is surprising to note the high number of operations exhibiting less than required drug efficacy, where nearly 1/3 of all operations fell below the 90% reduction level. In addition, these lower efficacies were not geographically restricted but widespread throughout the US including arid areas such as the northern Plains where anthelmintic usage has been relatively low.

The first documented case of resistance to macrocyclic lactones in the US was from a study initiated in 2004 in the upper Midwest (10). At that time, it was not known if this was an isolated incident resulting from excessive anthelmintic treatment, or an indication that anthelmintic resistance was on the rise in US cattle operations. The USDA NAHMS 2007–2008 beef study offered a unique opportunity to further evaluate this finding and clearly demonstrated that anthelmintic treatment failures were on the rise and widespread in the US and possibly North America. While "operator error" cannot be ruled out as a contributing factor to the apparent loss of anthelmintic efficacy, it is clear that macrocyclic lactones have become less effective in eliminating *Cooperia* sp. in the treated animals. A recent publication indicates that these parasites can have a significant negative impact on cattle productivity (29). If resistance to the macrocyclic lactones continues to expand in North American cattle as it has worldwide in sheep and in New Zealand cattle (4,5,7), this is only the beginning of a larger future problem. As indicated in the questionnaire that was a part of the cow-calf survey, internal parasites and anthelmintic resistance were selected as 2 of the top 3 most important issues facing the US beef industry (Ballweber et al, 2008, Colorado State University, personnel communication).

The results reported here should alert cattle producers to a number of important points: i) the efficacy of a producer's anthelmintic program must be routinely evaluated for efficacy, one can no longer assume that treatment equates to successful control; ii) producers should consider treating with multiple classes of drugs to achieve adequate parasite control; iii) pour-on formulations though easy to apply, provide the least effective level of nematode control, and therefore the best opportunity for development of resistant parasites; and iv) successful long-term and sustainable GI nematode control cannot be obtained by reliance only on anthelmintic treatment, sustainable nematode control must include good pasture management and animal husbandry.

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