UPDATE ON ANTHelmINTIC RESISTANCE IN GASTROINTESTINAL NEMATODE PARASITES OF CATTLE: HOW DOES THIS CHANGE OPTIMAL APPROACHES TO CONTROL?

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Beginning with phenothiazine in the 1950s, followed by the benzimidazoles in the 1960s, the imidazothiazole/tetrahydropyrimidines in the 1970s and the avermectin/milbemycins (AM) in the 1980s, a new class of anthelmintics was introduced into the marketplace each decade. This arsenal of highly effective and relatively inexpensive drugs led to recommendations for parasite control that were based almost solely on the frequent and strategic use of anthelmintics, the goals of which were to maximize livestock health, productivity, and profitability. Though this approach was highly successful for a number of decades, we are now experiencing ever-increasing levels of anthelmintic resistance in all drug classes, involving virtually all of the most economically important parasites of all livestock species. Resistance in parasites of cattle was slower to develop than in the small ruminant and equine sectors, but over the past decade we have seen a rapid escalation in the levels and distribution of anthelmintic resistance in parasites of cattle worldwide.

Though there are some published case reports of resistance in parasites of cattle in the US\(^1\),\(^2\), no studies have been performed to establish the national prevalence of resistance. Thus, we do not know how severe and widespread the problem is nationally. However, studies performed by my laboratory on a number of cow-calf farms in Georgia and on stocker cattle purchased at various stockyards in the southern region suggest that AM resistance in cattle is both common and widespread. In fact, we have not tested a farm in the last 5 years that did not have AM-resistant *Cooperia*, other than a single organic farm with a long history of no anthelmintic use. Resistance in *Cooperia* spp. and *Haemonchus* spp. are the most common, but we also have seen evidence in other species as well. Outside the US, there is a large amount of published data indicating that resistance is becoming a very serious problem; a study in New Zealand reported that ivermectin resistance was evident on 92% of cattle farms and resistance to both ivermectin and albendazole was evident on 74% of farms.\(^3\) More recently resistance in *Ostertagia ostertagi* has been found on numerous New Zealand beef farms. Very high prevalences of resistance have also been reported in studies performed in Brazil, Argentina and Australia.\(^4\)-\(^6\) *Cooperia* is consistently the species with the most resistance, but resistance in *Haemonchus* is also common. Resistance in *Oesophagostomum* and *Ostertagia* are reported less commonly, but recent evidence suggests increases in these species as well.

Historically *Cooperia* was not considered a very important pathogen. However over the past few decades, as a consequence of heavy use of AM drugs, the relative intensity of *Cooperia* compared to other species has risen substantially. Though *Cooperia* does not impact animal health and productivity to the degree that *Ostertagia* does, a recent study
confirmed that *Cooperia* infections do have a significant negative effect on growing cattle.\(^7\) So, although it may be unlikely to see clinically diseased cattle from *Cooperia*, there is little doubt that significant production losses can result from high levels of infection. Consequently, there is little evidence to support the opinion of some that AM resistance in *Cooperia* is not a major concern.

Luckily, resistance in *Ostertagia* is not yet a major problem in most of the world. However, recent evidence suggests that this might be in the process of changing. Should avermectin/milbemycin resistance emerge at high levels in *Ostertagia*, the problem of resistance in cattle parasites will reach a new level of importance and concern, as *Ostertagia* is a highly pathogenic species that can produce not only production loss, but also severe clinical disease and occasional deaths. The problem of anthelmintic resistance also needs to be viewed with an eye to the future. No new classes of anthelmintic have been introduced for use in cattle since ivermectin in 1981, 35 years ago. Other second generation AM drugs have provided some improvements since then, but AM resistance demonstrates a class effect; resistance to any one AM drug tends to confer resistance to all AM drugs. Additionally, no new novel classes of drugs have become available in the US over this time. The new drug monepantel (Zolvix®; Elanco) is sold throughout much of the world for sheep, but this drug has not yet been approved for use in the US, and it is unknown when or even if it ever will be. Currently there are no other new anthelmintic prospects in the late phase pipeline, thus, we are left in a situation where it could be a long while before a new anthelmintic class is sold for cattle. This makes it important that the efficacy of currently available products are protected as much as is reasonably possible.

Given this situation, the problem of anthelmintic resistance in parasites of cattle should not be ignored. Clearly, there is a great need for new research to address this issue, but waiting for this research before acting is not advisable. It is recommended that anthelmintic resistance in parasites of cattle be considered a major threat to cattle productivity, and that steps be taken to mitigate the potential problems resistance can cause. Because almost no research has been done in this area, no one can say for sure what are the best approaches to reduce the rate at which anthelmintic resistance evolves in cattle. However, it would seem logical to follow some of the recommendations for sheep, which are based on sound research. To do nothing seems irrational and short-sighted.

There are several approaches that have proven effective in reducing the rate with which resistance develops in sheep nematodes: (1) using drug combinations (two or more active compounds from different drug classes administered at the same time), (2) leaving the heaviest 10% of the flock untreated, (3) treating selectively based on some measure of parasitism or growth rate, (4) not treating the ewes and only treating the lambs.\(^8-10\) However, cattle are not sheep – thus some will be more difficult to implement in cattle, and some may be less effective in cattle. Still, some of these are easily adapted for cattle and are likely to be effective. For instance, not treating cows will not provide as much benefit as not treating ewes. This is due to differences between the species of importance and host-parasite interaction. In sheep, both ewes and lambs share the same parasite species in similar proportions, and periparturient ewes have relatively high EPG. Thus not treating the ewes provides a good level of refugia of the same species that infect the lambs. However, there is no periparturient rise in EPG in cattle, thus the relative level of egg shedding in cows remains low. Furthermore, cows usually have very good immunity to *Cooperia*, and are predominantly infected with *Ostertagia*, whereas calves are infected primarily with *Cooperia*. Consequently, not treating
cows will not have a major impact on the resistance levels in *Cooperia*. Still, this practice may be beneficial for slowing the development of resistance in *Ostertagia*, which is of growing concern. Quite simply – if *Ostertagia* develop resistance at high levels, the cattle industry will suffer great economic losses, and the health and welfare of cattle will suffer. Thus, implementation of any practices that can reduce this likelihood is a good idea, so long as it does not cause significant productivity losses now.

I have often heard people say that cattlemen will not leave some animals untreated since this will cause them productivity losses; it just goes against their common sense to not treat all cattle. This may sound reasonable at first, but how many of these same cattlemen are currently using anthelmintics that are poorly effective without knowing it? Based on my experience in testing cattle farms for resistance, this may be the majority of farms. So – yes they are treating all animals with the full associated costs, but they are not getting a highly effective result, and in some cases getting almost no benefit. I have tested several farms that had 0% reduction in FEC, and the cattlemen had not suspected resistance at all prior to the test. They would thus be much better off in the present and in the long term, if they tested the efficacy of drugs with a FEC reduction test (FECRT) to make sure they were using effective drugs.

Recent research has also demonstrated quite clearly that the use of anthelmintics in combination is a beneficial practice. In fact, in Australia and New Zealand there are few products sold as single actives; most products contain 3, 4, or 5 different anthelmintic classes (note that they have some anthelmintic classes that are not available in the US). There are 3 major benefits to using drugs in combination: (1) one gets an additive effect with each drug used, thus the efficacy of the treatment increases, sometimes dramatically (Table 1). (2) Provides broad-spectrum efficacy; resistance is species and drug specific, thus a second (or third) drug may kill any species resistant to the first drug. This will then return the broad-spectrum result that one aims to achieve (and that is specified on the product label). (3) By achieving a higher efficacy, there are fewer resistant survivors, thus there is a greater dilution of resistant worms by the susceptible portion of the population. For example, if 2 drugs each with 90% efficacy are used in rotation, then each time cattle are treated 10% of the worms (resistant) survive. In contrast if the 2 drugs are used in combination then the efficacy would be 99%; this yields 10X fewer resistant survivors (first drug kills 90%, second drug kills 90% of the remaining 10%).

Thus, cattlemen should perform a FECRT to determine which drugs are effective and then knowing this, they should optimally use 2 drugs in combination. Additionally, they should leave 10% untreated (selected from best looking animals; upper quartile) to provide un-treated refugia. Using this new approach, they will be getting a highly effective treatment in most of the herd, which will greatly diminish egg shedding thus reducing subsequent pasture contamination and re-infection. And by leaving some animals untreated they will be sustaining a drug-susceptible refugia, which will dilute out the small number of resistant worms that survive the treatment, thus maintaining a predominantly drug-susceptible worm population. The production loss in the 10% that are untreated is likely to be small because these were in the upper quartile of animals before the treatment, and so their growth was apparently not being heavily impaired by parasites. Studies in sheep comparing productivity of groups where 100% or 90% were treated demonstrated no significant differences in growth of lambs. By using this approach the overall herd productivity will be improved and the susceptibility of the worms to the drugs will be sustained much longer into the future. This approach is likely to be
even more effective when using new long-acting formulations, though research is needed to confirm this assumption.

In summary, given the current situation, it is recommended that cattle farms not assume high efficacy of their treatments and rather they should test for drug resistance using the fecal egg count reduction test (FECRT). Doing so will allow farms to make future treatment decisions based on the knowledge of which drugs are effective and which are not. Failing this, they should assume they have resistance and move immediately to the use of anthelmintic combinations. It is also important to appreciate that resistance develops slowly over many years and is undetectable during this time, but then suddenly reaches clinically detectable levels rapidly. Because the last phase of resistance development can happen quite quickly the FECRT should be repeated every few years. Currently there are no published standards for performing the FECRT in cattle, so there are different things being recommended by different veterinarians and parasitologists. This is a problem because an improperly designed or analyzed FECRT can result in erroneous conclusions. Thus it is important that clear guidelines for the FECRT in cattle be established; such guidelines are currently in preparation by the World Association for the Advancement of Veterinary Parasitology (WAAVP). Nevertheless, the guidelines below should closely resemble the final published protocol, thus I recommend the following procedures be used until such guidelines are published.

Table 1: Impact of using anthelmintics in combination on the efficacy of treatments:

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**RECOMMENDED (RMK) PROCEDURES FOR PERFORMING A FECRT IN CATTLE:**

- 15 animals per treatment group should be sampled
  - a minimum of 10 cattle should be used, but 20 is preferred when EPG are low
  - Each animal selected for testing should be uniquely identified
  - The same cattle must be sampled for FEC both pre and post treatment
- Cattle selected should be relatively uniform in age, breed, grazing history, anthelmintic exposure, other management considerations, etc.
- Weaned animals <16 months of age are preferable
  - FEC of adult cows are generally too low to perform a FECRT (see note below)
• If goal is to unequivocally detect drug resistance then only use injectable or oral formulations
  o pour-ons have high animal-to-animal variability and many sources of application error that can lead to reduced efficacy not due to resistance
• If goal is determine if the currently used pour-on product is effective then by all means it should be tested. However, border-line results should be interpreted carefully with regards to resistance.
  o If testing a pour-on it is critical that all cattle in the pasture be treated with the pour-on
    ▪ Normal licking behavior by cattle will cause drug transfer to any untreated animals, decreasing the dose absorbed by treated cattle
• Weigh each animal so proper dose is given
  o If each animal is not weighed and dosed individually, then all cattle should be dosed to the heaviest in the group.
  o Never dose to the average weight, as this results in ½ the animals being under-dosed
• Use label recommendations for dosage and application
• Ensure dosing equipment is calibrated and operating properly
• Fecal samples should be collected per rectum at time of Tx and 14–21 days post-Tx depending on the drugs tested.
  o Non-AM drugs (e.g. benzimidazoles or levamisole): 10-14 days
  o Ivermectin and other avermectin drugs: 14-17 days
  o Moxidectin: 17-21 days
  o If Non-AM and AM tested at same time then use: 14 days
  o Inserting two fingers into the rectum and gently massaging the dorsal rectum will stimulate most cattle to deposit feces into your hand; active or forceful collection is rarely needed.
• Data can be analyzed one of two ways
  1. Compare pre-Tx and post-Tx FEC of individual cattle
     • \[\frac{\text{PreTx FEC} - \text{PostTx FEC}}{\text{PreTx FEC}}\] *100
     • Then calculate the mean % reduction for the group
  2. Compare mean pre-Tx FEC and post-Tx FEC for the entire group of cattle
     • \[\frac{\text{Mean PreTx FEC} - \text{Mean PostTx FEC}}{\text{Mean PreTx FEC}}\] *100
     • This method is easier and is recommended unless a full statistical analysis is planned.
• Interpretation of egg count reduction
  o NOTE: due to many sources of variability the accuracy of results of FECRT are highly dependent on the number of animals tested and the number of eggs counted in the pre-treatment FEC. With few animals and low EPG there is a possibility of the observed results being different from the true situation.
  o To properly interpret results of a FECRT it is necessary to calculate 95% confidence intervals, however, to simplify interpretation the following guidelines can be used
    ▪ >95% = effective, no evidence of resistance
    ▪ 90-95% = reduced efficacy, suspected resistance in early stages
    ▪ 80-90% = reduced efficacy, highly suspicious of resistance
- <80% = ineffective, resistance is highly likely
  - Of course, efficacy data can only be correctly interpreted if the following are true: (1) cattle were treated with the proper dose, (2) cattle were treated using proper administration technique, (3) drug used was within the expiration date and was stored properly, (4) cattle were positively identified, (5) the same cattle were sampled both pre and post treatment, (6) fecal samples were labeled and stored correctly, (7) proper lab technique was used when performing FEC

- Use a FEC technique with sensitivity of 5 EPG or less
  - Mini-Flotac (and Fill Flotac) is my recommended procedure.
    - New method that resembles McMaster but provides a higher level of detection sensitivity and better precision
  - Modified Wisconsin can also be used, but testing in our lab shows it is both more time consuming and less accurate than the mini-Flotac
  - NOTE: FEC sensitivity that is required will depend on the magnitude of the EPG in the group being tested.
    - For the most accurate result it is recommended that at least 200 eggs be counted pre-treatment. However, so long as at least 100 eggs are counted, results will be accurate more often than not.
    - Thus if using a technique with 5 EPG detection, then the mean EPG will need to be 100 if 10 cattle are tested and 65 is 15 cattle are tested. If mean FEC is <50 EPG, then there are 3 options:
      a. Use a procedure with a more sensitive detection level.
      b. Repeat the FEC or read an additional chamber for each animal
      c. Test more animals
  - If mean FEC are >500 EPG (group size treated = 10 cattle) or >250 EPG (group size treated = 20 cattle), then a McMaster can used.
  - Adult Cattle: adult cattle tend to have very low EPG, often averaging less than 2 EPG. Thus, it is extremely difficult to perform a FECRT in adult cattle with accuracy unless many cows are tested and a very sensitive FEC method (1 EPG or less) are used.

REFERENCES


