DEFINING PARASITE RESISTANCE TO ANTHELMINTICS

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Summary
Anthelmintic resistance involves genetic changes in parasite populations in response to ongoing drug exposure. Detection can only be accomplished by rigorously documenting efficacy reductions in the same hosts treated with the same drug at the same dose rate, against the same worms. A diversity of other “nonresistance” factors can impact the efficacy of deworming products used for parasite control in cattle, and these factors can easily be confused as resistance. Effective treatment programs must address such nonresistance issues to ensure anthelmintics are correctly deployed and high efficacy is achieved, and to avoid unfounded fears of resistance when other factors should be implicated in episodes of reduced efficacy.

Key points
• Resistance of cattle parasites to anthelmintics is a concern since effective deworming products are vital tools for raising healthy, productive animals.

• While the development of anthelmintic resistance is certainly possible and has been demonstrated, multiple other nonresistance factors have been identified that also can erode the efficacy of deworming products.

• Reduced efficacy caused by nonresistance issues often is confused as resistance, leading to unfounded concern that might be alleviated by carefully addressing these other factors that can impact treatment success.

• No comprehensive scientific database exists regarding the scope of parasite resistance to anthelmintics in U.S. cattle populations.

• Implementation of treatment protocols recommended by Pfizer Animal Health can help ensure optimal benefits are derived from parasite control programs.
The use of anthelmintics in cattle production is a standard management practice that yields tremendous financial benefits. Cattle producers typically assume the deworming treatments they administer are effective for controlling parasites in their herd, and this is usually the case. However, occasional instances of reduced efficacy do occur, and such events often prompt immediate concerns about drug resistance when, in fact, numerous factors may actually be the actual cause of the episode. Still, the potential for anthelmintic resistance is always a possibility, so consideration is warranted of characteristics that might comprise true parasite resistance.

**Defining resistance**

Anthelmintic resistance may be defined as the lack of drug efficacy in a population of helminth parasites that was previously sensitive to the drug at a defined dose and in a defined host. This definition implies that resistance is an acquired genetic change in the population due to mutation and/or selection during repeated use of the anthelmintic. As a result, the drug no longer performs as it previously did under the same circumstances (same type of cattle, same type of infection, etc.). Resistance can be detected via reduced drug impacts on fecal egg count reduction and/or reduced parasite count reduction. Whether the efficacy reduction is just barely detectable (i.e., 1 percent to 3 percent of previous efficacy levels) or clinically measurable (i.e., 30 percent to 40 percent less efficacy), both cases are still considered resistance.

However, reductions in drug efficacy can be caused by factors other than acquired genetic resistance (nonresistance events). For example, a drug administered at a recommended dose may lack efficacy against a particular parasite species, but nothing changed over time since an acceptable level of efficacy (greater than or equal to 90 percent) had never been demonstrated at that dose against that species [such as efficacy of DECTOMAX® against *Nematodirus helvetianus* in cattle]. This scenario is considered nonresistance because the less-than-optimal efficacy did not involve changes in the genome of the parasites.

Other nonresistance factors may be implicated when reduced efficacy is observed with a product previously shown to work well at the same defined dose against a population of parasites in the same host. Possible nonresistance causes of efficacy reduction include:

1. Underestimation of animal weight, thus underdosing of that animal
2. Poor absorption of drug, especially with topical products
3. Topical drug washed off or licked off the treated animal
4. Drug concentration in the container/bottle lower than the label states
5. Animal missed in the chute
6. Animal has hyperactive drug metabolic system
7. Animal has very high or very low proportion of body fat
8. Product applied incorrectly — e.g., pour-on applied as spot-on; pour-on applied to deer without raising guard hairs
9. Product applied when too cold to be syringed or absorbed (viscosity too high)
10. Reinfection confused with lack of efficacy
11. Misdiagnosis — e.g., free-living nematode eggs or protozoal cysts in feces
12. Tolerance — e.g., *Cooperia* (dose-limiting parasite for macrocyclic lactones)

**Resistance mechanisms**

Anthelmintics commonly used in cattle production belong to three drug classes:

- Imidazothiazoles — e.g., levamisole
- Benzimidazoles — e.g., albendazole, fenbendazole, oxfendazole
- Macrocyclic lactones (ML) or avermectins and milbemycins (endectocides) — e.g., abamectin, doramectin, eprinomectin, ivermectin, moxidectin

All three classes have been implicated for extensive internal nematode parasite resistance in sheep and goats, but only infrequent resistance events in cattle, swine and horses. The parasite genera that are generally involved in ruminants are *Haemonchus*, *Cooperia*, *Ostertagia* and *Trichostrongylus*. Reduction of drug efficacy against *Haemonchus* in sheep and goats is traditionally viewed as the first sign of possible resistance. Several interacting issues are thought to contribute toward drug resistance in sheep and goats: 1) frequent treatment in this host-parasite complex; 2) plasticity of the *Haemonchus*
genome; 3) underdosing of goats, which, because of their more active enzymes for metabolizing drugs, require about twice the dose as sheep (most labels recommend the same dose for both types of small ruminants).

Four well-defined mechanisms of resistance are generally recognized:

- **Site-resistant mutant** — the binding site of the drug, or an area that impacts the binding site, is changed through mutation and/or selection through repeated drug use so it no longer binds the drug
- **Metabolic-resistant mutant** — the drug metabolic system of the parasite is activated to break-down the drug so fast that a lethal concentration does not reach the active site in the parasite
- **Competitive-binding mutant** — the parasite produces a high concentration of a nonspecific protein in the cell that binds the drug before it gets to the active site (e.g., glutathione reductase, glycoprotein P)
- **Behavioral mutant** — the parasite moves to a site of low drug concentration, staying there until the drug leaves the body

Anthelmintic resistance in cattle has developed much slower than resistance in sheep or goats due to less frequent dosing of cattle. Climate also has an impact; resistance in cattle develops even more slowly in the United States and Canada compared with tropical and subtropical cattle-producing countries around the world. In North America, lower stocking densities are typically employed, and weather patterns often include one or two periods (hot-dry and cold-dry) that serve to kill nematode eggs and larvae on pasture.

### Resistance/nonresistance reports

Reports of imidazothiazole (levamisole) and benzimidazole resistance in U.S. cattle began appearing in the scientific literature 10 to 20 years after launch of these drug classes during the mid-1970s. However, no extensive epidemiological database exists to determine the extent of these resistance problems.

Macrocyclic lactone products first became available in the early 1980s, prompting a dramatic reduction in use of levamisole and benzimidazoles. With the launches of the various ML products (ivermectin in cattle in 1981, eprinomectin in 1995, DECTOMAX in 1996, moxidectin in 1999), most cattle producers have relied heavily or completely on this class of compounds. In recent years, reduced efficacy of benzimidazoles and avermectins have been reported from Southeastern grazing cattle.\(^1\) Worms that remained following ML treatments were primarily *Cooperia* (the rate-limiting parasite for ML) and *Haemonchus*; parasites that remained after benzimidazole treatments were *Haemonchus*; and a few *Ostertagia* were observed after levamisole treatments.

A report of nonresistance that many have been misconstrued as ML resistance also has been published relatively recently (2004).\(^4\) Researchers compared the efficacies of five ivermectin treatments in grazing calves in Arkansas with cattle that remained untreated. The medicated treatment groups included branded Ivomec\(^{®}\) (ivermectin) pour-on and four other generic ivermectin pour-on products. Efficacy was assessed by weight gains and fecal egg count reductions (FECR) monitored weekly over eight weeks. The branded Ivomec pour-on generated 96 percent FECR at Day 14 post-treatment and 56 percent FECR at Day 56. These results exactly matched the expected efficacies as compared with data from similar trials conducted in 1984 when Ivomec pour-on was launched. However, the four generic ivermectin pour-on products had FECR of 81 percent to 93 percent at Day 14, and only 1 percent to 38 percent FECR at Day 56, much below expected FECR. The 4 percent of eggs left by the treatment of branded Ivomec pour-on were all *Cooperia*, as determined by larval cultures (*Cooperia* is the rate-limiting species for the ML products and was not completely eliminated from cattle in the initial trials of Ivomec). Weight gains for cattle treated with the branded Ivomec product averaged 0.06 to 0.21 pound per day greater than the untreated or generic ivermectin pour-on treatment groups.

These study results suggest two conclusions: 1) in this study, no resistance to branded Ivomec pour-on was detected; and 2) the generic pour-on products demonstrated an undefined nonresistance problem.

### Examples of nonresistance

In the context of the list of nonresistance issues discussed earlier, several potential pitfalls emerge that must be avoided if true resistance is to be detected. Examples include:

1. **Underdosing**: Although the number of 1,100-pound cows is much lower in the United States today compared with 30 years ago, producers still tend to dose cows at the 1,100-pound dose.
even if the animal weighs 1,800 pounds. Weighing animals and dosing to the exact weight is often overlooked, to the detriment of anthelmintic efficacy. Furthermore, if cattle are weighed, the weights are often averaged, resulting in underdosing half the animals while too much drug is given to the other animals.

2. Poor absorption: Pharmacokinetic research studies have shown that only about 15 percent to 20 percent of a pour-on drug achieves delivery into the bloodstream of a clean, dry, short-haired 500-pound animals. Thus, the amount of drug entering the body of a dirty, wet, long-haired 800-pound animal would likely be insufficient.

3. Topical drug washed/licked off: Research has documented a 36 percent increase in blood levels (AUC) of a pour-on ML product when animals lick or self-groom after treatment, with 10-times more drug in the feces. Since grooming behavior cannot be controlled, the actual effective dose rate being delivered is unknown.

4. Low drug concentration: Stability of anthelmintics (especially ML products) is based on absence of oxygen and protection against ultraviolet light. If a bottle of an ML product is stored in the store or veterinary office without an effective oxygen barrier or ultraviolet light block, or the bottle is opened and half of the drug is used, the actual amount of effective product in that bottle is unknown.

5. Animal missed in the chute: If a treatment is overlooked, the animal obviously receives no parasite protection.

6. Hyperactive metabolism: Detoxifying enzymes in the liver are activated in cattle exposed to toxic weeds, to handle the toxin(s). When an animal has hyperactive metabolic enzymes, the elevated rate of anthelmintic breakdown basically reduces dose delivery to the active site of the parasite. One exception to this principle is that ML products appear to help eliminate the toxic effects of endophyte-infested fescue; drugs of other anthelmintic classes have not been reported to induce a similar effect.

7. Body fat: If an animal has a very low proportion of body fat, fat-soluble ML products cannot be stored in the fat and released slowly. An excellent therapeutic effect would be predicted, but with a reduced degree of persistent activity. Conversely, if an animal has very high proportion of body fat, the ML would tend to rapidly disperse from the bloodstream and stay in the animal for an increased time, which would potentially be reflected by lower therapeutic efficacy but longer persistent activity.

8. Wrong application: If a pour-on product intended for application along the entire backline is poured in one spot (“spot-on”), the amount of drug absorbed through the skin will be greatly reduced. Also, if the animal is treated for lice on one spot and the lice then move to the opposite end of the animal, drug exposure will again be reduced. Treatment of deer requires raising of the guard hairs or the product will simply run off the back. The pouring process for deer must go from back to front, with a hand just in front of the dosing gun raising the guard hairs.

9. Cold weather: If ML products are poured on cattle at 20°F, the viscosity of the product may reduce absorption. This is particularly true of eprinomectin and moxidectin (viscosity values of 40 and 101, respectively), which have little or no alcohol in their pour-on carrier formulations. In contrast, Ivomec and DECTOMAX pour-ons contain 75 percent isopropanol, which acts like antifreeze (viscosity of 8.4 and 8.7, respectively) during syringing and then evaporates on the skin, creating heat of evaporation that helps drive drug molecules through the hair follicles.

10. Reinfection: If cattle are treated and returned to infected pastures, even if all parasites are removed, adult Cooperia can be found by 18 days post-treatment, with egg-laying adults of other species by Day 21. If FECR is the measure of treatment efficacy, only a small depression in egg counts may be achieved by levamisole or benzimidazoles by Day 22. Since ML products have one to four weeks of persistent activity (depending upon product and parasite species), a longer time period would pass before egg-laying adults develop. Another problem arises with benzimidazoles and levamisole if inhibited larvae are inhabiting the tissues. Since inhibited larvae will not be killed by these products, parasites will emerge from tissues after treatment and egg-laying adults will develop within seven to 10 days post-treatment.
11. Misdiagnosis: Free-living roundworms in the soil feed on the bacterial fauna living in cattle manure at high concentrations. If a fecal pat from the ground is used to measure egg counts, a misdiagnosis is imminent if the examiner does not know how to distinguish free-living nematode eggs from parasitic nematode eggs. Certain protozoal cysts also can resemble nematode eggs, offering another source of misdiagnosis.

12. Tolerance: Tolerance is a term applied to *Cooperia* in regard to ML treatments. Invariably, 1 percent to 5 percent of any *Cooperia* population will not be killed by a full dose of an ML product. This percentage was noted at the time of original testing of the products in the 1980s and that percentage generally has neither increased nor decreased. Such “tolerance” does not qualify as “resistance” because it was known to exist when the products were originally developed and no subsequent genetic changes have ensued.

**Treatment recommendations**

No extensive body of data for anthelmintic resistance in U.S. cattle currently exists. Until such a database becomes available, parasitologists really have no idea if resistance is a problem just beginning or if it is already approaching a clinically significant level. A geographically relevant survey is needed involving FECR tests on a large number of cattle plus data generated from a smaller number of animals from the same herds, and the survey also must include necropsy and worm-count data for both adult and larval forms of the parasites.

Before such a data set becomes available (if ever), general recommendations should include the following considerations:

1. Use generic ivermectin products cautiously. An unknown nonresistance problem has been described in the literature but it is not presently understood.

2. Prefer injectable products to pour-on products when possible. The amount of product delivered to the bloodstream is greater with injectables, and the animal-to-animal variation is much smaller with injectables.

3. If treatment of calves, stockers or feeders does not result in a FECR of greater than 80 percent and/or the cattle do not respond with an enhanced rate of gain, rotate to a new class to see if better efficacy and productivity responses are achieved.

4. If resistance is suspected in feedlot cattle, a combination of two classes appears to be an economically viable alternative. However, use of combinations in grazing situations is discouraged because selection for double-resistant mutants may be enhanced if resistance to one of the products in the combination is present.

5. Encourage pharmaceutical companies and academic researchers to accelerate their searches for a new classes of anthelmintics since effective products are vital tools for raising healthy, productive animals.

More often than not, instances of reduced anthelmintic efficacy (as well as parasitcides in general) are impacted by factors other than resistance or drug activity failure. Simply put, proper use of anthelmintics, at the proper time of the season, is critical in ensuring effective parasite control. Treatment recommendations to help ensure optimal benefits are derived from a sound parasite control programs which can include the following Pfizer Animal Health products:

- Treat cattle with DECTOMAX Injectable in the spring
- Treat cattle with VALBAZEN® and/or DECTOMAX Pour-On in the fall
- For producers in Gulf states, northwestern Oregon, Washington or California, treat cattle with VALBAZEN and/or DECTOMAX Pour-On in the spring and DECTOMAX Injectable in the fall
- Treat feedlot cattle with DURASECT® II Pour-On for lice breaks
DECTOMAX Important Safety Information: DECTOMAX Injectable has a 35-day pre-slaughter withdrawal period. DECTOMAX Pour-On has a 45-day pre-slaughter withdrawal period. Do not use in dairy cows 20 months of age or older. DECTOMAX has been developed specifically for cattle and swine. Use in dogs may result in fatalities.

VALBAZEN Important Safety Information: Cattle must not be slaughtered within 27 days after the last treatment with VALBAZEN. Not for use in lactating dairy cattle. Do not administer to female cattle during the first 45 days of pregnancy or for 45 days after removal of bulls.