Basis of Anthelmintic Resistance and Novel Approaches to Development of New Efficacious Anthelmintic Drugs

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Current Anthelmintics
3 Classes of anthelmintic drugs registered in the USA:

1.) Benzimidazoles
   - Fenbendazole, Safeguard, Panacur

2.) Macrocyclic Lactones
   - Avermectins: Ivermectin, Ivomec, Primectin, Privermectin
   - Eprinomectin: Eprinex
   - Doramectin: Dectomax
   - Milbemycins: Moxidectin, Cydectin, Quest

3.) Nicotinic Agonists
   - Imidothiazoles: Levamisole, Prohibit
   - Tetrahydropyrimidines: Morantel, Rumatel, Positive Goat Pellet, Goat dewormer, Pyrantel, Strongid

How do anthelmintic drugs kill parasites?

- Benzimidazoles (Valbazen, Safeguard): Bind to a parasite protein called β-tubulin leading to collapse of parasite skeleton structure.

- Avermectin/Milbemycins (Ivomec, cydectin): Bind to proteins in throat (pharynx) of parasite leading to paralysis – parasite can’t eat anymore & dies of starvation!

- Imidazothiazoles/Tetrahydropyrimidines (Levamisole, Pyrantel, Morantel): bind to acetylcholine receptors causing muscle paralysis.
Status of Anthelmintics Efficacy

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Generic Name/Brands</th>
<th>Parasite Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzimidazoles:</td>
<td>Thiabendazole, Albendazole</td>
<td>High prevalence of resistance</td>
</tr>
<tr>
<td></td>
<td>Sheep, goat, Horse</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1961, 1964</td>
<td></td>
</tr>
<tr>
<td>Imidazothiazoles-heterocyclics</td>
<td>Levamisole, Pyrantel</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep, Horse</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1970, 1979</td>
<td></td>
</tr>
<tr>
<td>Macrocyclic lactones:</td>
<td>Ivermectin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep, Horse</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1981, 1988</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moxidectin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep, Horse</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1991, 1995</td>
<td></td>
</tr>
<tr>
<td>Amino-acetonitriles:</td>
<td>Monepantel</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2009, 2013</td>
<td></td>
</tr>
</tbody>
</table>

**Anthelmintics Efficacy in Small Ruminants**

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Generic Name/Brands</th>
<th>Parasite Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzimidazoles</td>
<td>Albendazole (Valbazen®, Fenbendazole (Safeguard®))</td>
<td>High prevalence of resistance</td>
</tr>
<tr>
<td>Ivermectin/ MIlbemycins</td>
<td>Ivermectin (Imurom®)</td>
<td>High prevalence of resistance</td>
</tr>
<tr>
<td></td>
<td>Moxidectin (Cydectin®)</td>
<td>High prevalence of resistance</td>
</tr>
<tr>
<td>Imidazothiazoles/Tetrahydropyrimidine</td>
<td>Levamisole (Timbloc®), Pyrantel (Strongid®), Norantel (Banex®)</td>
<td>High prevalence of resistance</td>
</tr>
</tbody>
</table>

Challenges

• Increased helminth pressure due to climate & environmental changes will lead to increased anthelmintics use & subsequent selection for stronger resistance

• Poor financial incentives to big pharma for new anthelmintic development
What is Drug Resistance?

- Ability of parasites in a population to survive drug treatments that are generally effective
- Drug treatment eradicates worms that are susceptible to that particular drug
- However, resistant parasites survive and pass-on "resistance" phenotype and/or genotype to daughter parasites

Selection for Drug Resistance

What Causes Drug Resistance?

1) Frequent deworming
   - Treating on a schedule
   - Treating whole flock
2) Use of sub-optimal dosages
   - Giving insufficient dose (by weight)
   - Injecting dewormers
   - Pour-on dewormers
   - Putting dewormers in mouth instead of over tongue
   - Use of expired drugs
3) Incorrect use of drugs, storage
4) Continuous use of same class of drug
5) Treating animals when infection levels are low
6) Any management practice which increases the need for deworming
Anthelmintic Resistance is Genetic

- Resistant worms pass their resistant genes onto offsprings: resistance is permanent
- Resistance cannot be prevented but can be slowed down

Practices that help slow development of drug Resistance

- Targeted, selective treatment
- Leaving some animals untreated
- Dosing based on accurate weight
- Depositing drug over tongue
- Leaving treated animals in dry lot or barn for 48 hours
- Fasting animals when using benzimidazoles or avermectins

Targeted Selective Treatment (TST)

- Treating only animals that require or will benefit from the treatment
- This reduces the use of anthelmintics and maintains some animals in-refugia (animals with worms unexposed to drug)
- This approach slows development of anthelmintic resistance
Deciding what Animals to Treat

• Requires practical decision making tools:

1. FAMACHA:
   • Useful for blood sucking parasites infections
   • Examine animal in good natural light
   • Open eyelid as shown in picture for a short time only
   • Observe color inside eyelid
   • Compare eyelid color to FAMACHA card to obtain a score of 1-5 (1 = not anemic; 5 = severely anemic)

2. Five Point Check: an extension of FAMACHA:
   • Unlike FAMACHA, this system is useful for assessing animals in need of treatment against all types of parasites that commonly affect ruminants
   • Checks for five points on the animal: eyes, jaw, back, tail and nose.

Body Condition Scoring Goats

<table>
<thead>
<tr>
<th>Score</th>
<th>Spineous processes</th>
<th>Rib cage</th>
<th>Loins eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very thin</td>
<td>Easy to see a few, sharp</td>
<td>Skin fat covering</td>
</tr>
<tr>
<td>2</td>
<td>Thin</td>
<td>Easy to feel, but smooth</td>
<td>Smooth, some fat cover</td>
</tr>
<tr>
<td>3</td>
<td>Good condition</td>
<td>Smooth and rounded</td>
<td>Smooth, bone fat cover</td>
</tr>
<tr>
<td>4</td>
<td>Fat</td>
<td>Can feel with firm pressure, no points can be felt</td>
<td>Individual ribs cannot be felt, but can still feel between ribs</td>
</tr>
<tr>
<td>5</td>
<td>Obese</td>
<td>Smooth, no individual vertebra can be felt</td>
<td>Thicks fat cover, may be knobby and &quot;tuffy&quot;</td>
</tr>
</tbody>
</table>

Source: www.smarthouse.info
Checking for Anthelmintic Resistance

- It is recommended that you check for anthelmintic resistance every 2-3 years.
- There are two ways to test for anthelmintic resistance:
  1. Fecal egg count reduction test (FECRT)
  2. DrenchRite Assay

**Fecal egg count reduction test (FECRT)**

- Compare pre- and post-treatment fecal egg counts:
  a) 8-10 days for benzimidazoles (SelcoGuard®, Vibenox®)
  b) 14-21 days for macrocyclic lactones (Ivermectin®, Cydectin®)
  c) 5-7 days for levamisole (Hardrive®)
  d) 10-14 days for all dewormers

Fecal Egg Counting By Modified McMaster Procedure

**Materials and Reagents:**
- Microscope (10 x 10 = 100x)
- McMaster slide
- Floatation solution
- Scale
- Cups or vials
- Tongue depressors
- Cheese cloth or tea strainer
Modified McMaster Method:
1. Weigh 2 g of freshly collected feces (if storing feces, refrigerate to avoid hatching of worm eggs)
2. Add 28 ml of flotation solution
3. Crush and mix feces using tongue depressor
4. Drain solution through cheese cloth or tea strainer into a clean cup
5. After stirring solution, draw up solution from top of mixture
6. Fill both sides of slide chamber
7. Allow slide to sit for 5-10 minutes
8. Place slide on microscope and focus grids
9. Count strongyle-type eggs inside of under grid lines in the entire chamber
10. Record total number of eggs in both chambers
11. Multiply the sum by 50 to get Eggs Per Gram of feces (EPG)
Parasites vary in their fecundity (egg laying capacity).
Immature worms (L4) do not lay eggs but still harm the animal.
Variability in counts from day-to-day.
Eggs are not evenly distributed in manure.
Loose stools (diarrhea) may lead to underestimated egg counts.
Some eggs cannot be differentiated from others.
Not all parasite strains are pathogenic.
Varying procedures for doing fecal egg counts.
Possible human error.

**Fecal Egg Count Limitations**

**2) DrenchRite® Assay**

- Determines drug resistance for all anthelmintic classes simultaneously from a pooled fecal sample.
- However, resistance to Cedecono™ is predicted based on the results for ken nectin.
- Also determines which parasites your animals have.
- Collect a pooled fecal sample from at least 10 animals with ≥500-5000 eggs.
- Follow instructions for collecting, handling, and shipping sample to Dr. Ray Kaplan's lab at the University of Georgia.

**DrenchRite Report Form**

**Client Information**

- Client Name: [Blank]
- Treatment Date: [Blank]
- Reference: [Blank]
- Cost: [Blank]
- Frequency: [Blank]
- Comments: [Blank]

**Part Results**

- Procedural or treatment parameter present: [Blank]
- Temperature: [BLANK]
- Color: [BLANK]

<table>
<thead>
<tr>
<th>Medication</th>
<th>LS</th>
<th>LL</th>
<th>OL</th>
<th>MR</th>
<th>DE</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DrenchRite</td>
<td>9.5</td>
<td>4.5</td>
<td>5.5</td>
<td>7</td>
<td>[Blank]</td>
<td>[Blank]</td>
</tr>
</tbody>
</table>

**Interpretation**

- Resistance to treatment: [Blank]
- Procedural or treatment parameter present: [Blank]
- Temperature: [BLANK]
- Color: [BLANK]

**A comparison of tests**

**FECRT**

- Takes 7-14 days to get results, longer if someone else does FECR.
- Cost for 75 samples:
  - 75 x labor = [Blank]
  - 75 x $5 = $375
  - 75 x $10 = $750
- Need more animals
- Get percent resistance/efficacy instead of R, S, and SR
- Results can vary by animal.

**DrenchRite® Assay**

- Labor intensive lab test
- Only one lab in US does DrenchRite® Test (UGA)
- Takes 3-4 weeks to get results
- Cost $450 per sample

**Understanding anthelmintic resistance**

- 95-100% effective, small number of resistant worms may be present.
- 80-90% effective, treatment is effective, but resistance is increasing.
- Less than 80%, production issues become apparent as effectiveness of dewormer is seen close to zero.
- Anthelmintic failure, Animals die.
What you can do when deworming is not effective

1. Dose with another class of anthelmintic
2. Give supportive therapy
   - Vitamin B complex
   - Iron or red Cell
   - Nutri-drench
   - Probiotics
   - Proteinaceous feeds
3. Remove parasitized animal from pasture (source of re-infection)

Management Practices that Predispose to Anthelmintic Resistance Development:

1. Overstocking
2. Overgrazing
3. Susceptible animals/breeds
4. Poor nutrition
5. Poor pasture quality
6. Poor sanitation

Good Management Practices:

1. Pasture rest/rotation
2. Good nutrition, especially protein
3. Clean pastures
4. Annual Pastures
5. Mowing and haying
6. Tilling
7. Multispecies grazing
8. Mixed pastures (with legumes)
9. Browsing/taller forages
10. Tanniferous forages
11. Resistant animals/breeds

Advances being made

- Generation of new combinations/dosing regimens for existing drugs
- Biological approaches:
  - high protein plants (chicory)
  - plants with condensed tannins, polyphenols with direct effect on nematodes
  - Feeding nematode-trapping fungus (Duddingtonia flagrans)
  - Dietary supplementation with urea
- Identification of novel drug targets: phospholipid biosynthetic pathways
Phospholipid biosynthetic Pathways as Potential Anthelmintic Drug Targets in Nematodes

- Phospholipids are important structural and functional components of cell membrane for eukaryotic cells (Sood, 2006)
- Phosphatidylcholine and Phosphatidylethanolamine accounts for majority of the eukaryote membrane phospholipids (Lee and Jez, 2014)

Phosphoethanolamine Methyltransferase (PMT) is a Critical Enzyme in Biosynthesis of Phosphatidylcholine

Concurrent knockdown of HcPMT1 & HcPMT2 attenuates Phospholipid content & decreases viability of H. contortus

(Witola et al., 2006, 2007, 2008; Bobenchik & Witola et al., 2013; Witola et al., 2016a)
Fluorescence-based SAM-dependent Methyltransferase Assay

HcPMT1 & 2 catalyze SAM-dependent methylation of Phosphoethanolamine

Witola et al., 2016b

HcPMT1 & 2 catalyze SAM-dependent methylation of Phosphoethanolamine

Witola et al., 2016b
NSC-641296 Possesses Anthelmintic activity against L3 & adult H. contortus

Future Studies

- Characterize putative PMTs from different families of livestock nematodes (Chabertiidae, Trichostrongylidae, Dictyocaulidae, Ancylostomatoidae and Ascarididae) & identify broad-spectrum inhibitors.

- Test candidate inhibitors’ in vitro & in vivo anthelmintic efficacy against mixed species & multi-drug-resistant nematodes.

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- Dr. Ray Kaplan, DVM, Ph.D., UGA
- Dr. Albert Russell, Ph.D., Tuskegee University
- Dr. Byeng Min, Ph.D., Tuskegee University
- Dr. Peter Yau, Ph.D., UIUC
THANK YOU!