

Anthelmintic Resistance And The Changing Landscape of Parasite Control

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Anthelmintic Resistance Status 2006

- Considered a major threat to the current and future control of nematode parasites of ruminants and horses
 - Worldwide phenomena
 - All hosts
 - All drug classes
 - Almost all major parasites
 - The prevalence of **multi-drug resistant** nematodes in sheep, goats and horses is extremely high

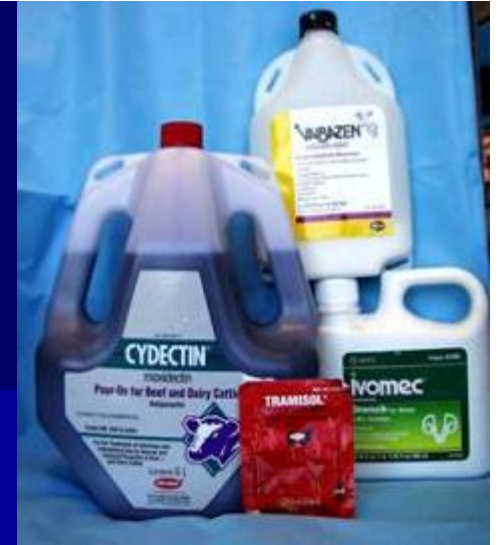
Anthelmintic Resistance Background to the Problem

- Age of modern anthelmintics
 - Effective, broad-spectrum, cheap, safe
- Lots of new data on parasite epidemiology
 - Parasitologists & Pharmaceutical Co. encouraged preventative treatment
 - resistance issues were ignored
- Over-reliance on anthelmintics
 - Loss of common sense approaches
 - Belief there will always be a new drug



Anthelmintic Classes

Nematocides



■ Benzimidazoles

- fenbendazole (FBZ), oxibendazole (OBZ), albendazole (ABZ), mebendazole (MBZ), others

■ Avermectin / Milbemycins

- ivermectin (IVM), eprinomectin (EPR), doramectin (DRM) moxidectin (MOX), others

■ Imidazothiazoles / Tetrahydropyrimidines

- levamisole (LEV), pyrantel (PYR), morantel (MOR) others

We Are At Risk Of Having No Effective Anthelmintics In The Near Future

- Large drug companies invest in drugs with very large profit potential
 - little investment in new animal drugs
 - "ivermectinization" of anthelmintic development
- Reverse pipeline for anthelmintics
 - Veterinary medicine (particularly cattle) is primary market
 - Must be inexpensive to synthesize

We Are At Risk Of Having No Effective Anthelmintics In The Near Future

- New drug classes introduced every decade during 50's, 60's, 70's, and 80's
 - Less than 20 years between TBZ and IVM
- No new drug classes introduced to the market from 1981 to 2005
 - "We have what we have"

Where Are The New Drugs ???

- Cyclodepsipeptide (emodepside) introduced for cats in 2005
 - Bayer Animal Health
- Resistance is very likely to outpace the introduction of new anthelmintics
 - 13 years from first published report of cyclodepsipeptide as a new anthelmintic to marketing of a product
 - New anthelmintics that are developed may be much more expensive



Anthelmintic Resistance:



- The ability of worms in a population to survive drug treatments that are generally effective against the same species and stage of infection at the same dose rate
 - Caused by changes in allele frequencies of "resistance" genes
 - Resistance Genes = alleles of relevant genes that confer resistance
 - Result of drug selection

Where Do Resistant Worms Come From ???

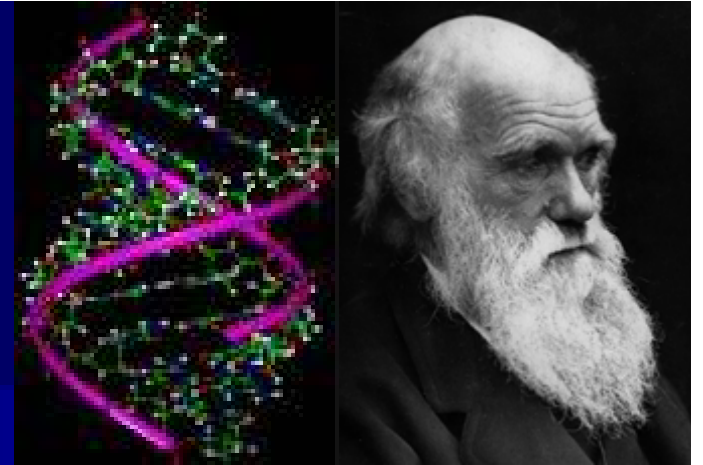
- Nematodes have great genetic diversity & large pop'n sizes
 - High mutation rates and rapid evolution
 - *Haemonchus contortus*
 - 5000 eggs per female/day
 - 500 female worms/animal
 - 50 animals → approx 1 billion eggs per week



Where Do Resistant Worms Come From ???

- “Resistant” worms exist within populations prior to the introduction of a drug
 - Same allele seen in wide variety of resistant lines
 - R-allele arose once and spread as neutral allele
 - Initial allele frequency is very low
 - Relative changes in allele frequencies rather than appearance of new alleles

Development of Resistance



- Resistance is an inevitable consequence of drug selection on helminth populations
 - Treatment eliminates parasites whose genotype renders them susceptible
 - Parasites that are resistant survive and pass on their "resistant" alleles
 - High level of animal movement disperses resistant worms



Detection of Drug Resistance

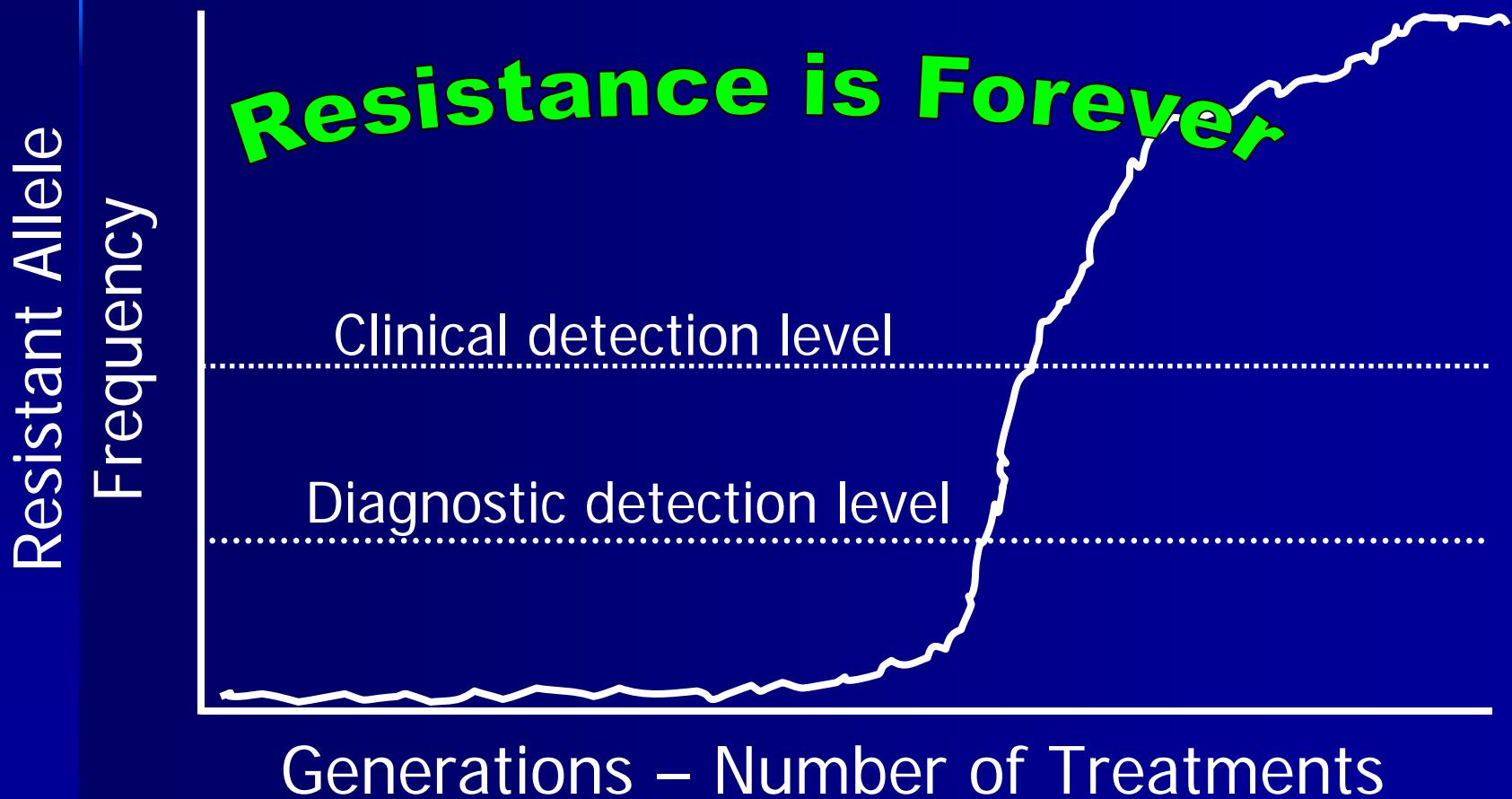
■ Clinical definition of resistance

- when normal therapeutic dose is no longer fully effective
- <95% reduction in FEC
- many worms still killed

■ Phenotypic trait

- Genotypic resistance occurs long before
- Drug resistance exists long before it can be detected clinically

Changes in Allele Frequency in Response to Drug Selection



Development of Resistance: Nematodes Vs. Non-Metazoan Organisms

- Nematodes reproduce sexually
 - Resistant worms cannot directly multiply themselves
- R-offspring must infect a new host
 - No direct infection from 1 host to the next
 - All helminth parasites have a free-living (non-parasitic) stage or utilize an intermediate host
 - Eggs shed from resistant worms are greatly diluted by those of susceptible worms
 - New hosts are infected 1 worm at a time

Development of Resistance: Nematodes Vs. Non-Metazoan Organisms

- With nematodes, re-infection and drug selection must occur over many life-cycles to increase the frequency of resistant worms to clinically important levels
 - In early stages, large majority of worms are not resistant – chances of R x R matings is low
 - Resistance occurs slowly over years
- This contrasts greatly with organisms that can reproduce clonally
 - 1 surviving resistant organism can replicate itself and repopulate the host
 - New host is infected with a “pure” resistant strain

Resistance is Inevitable

- Natural biological consequence of drug treatment

■ What Can We Do ???

- Rate of selection for resistance can be greatly reduced
- Preserve drug efficacy for as long as possible
 - Selective treatment
 - Increase refugia



Refugia



- The proportion of the population that is not under selection by drug treatment
 - “In Refuge” from drug
 - Worms in untreated animals
 - Eggs and larvae on pasture
- Provides a pool of sensitive genes
 - Dilutes frequency of resistant genes
- Now recognized as the most important component of drug resistance selection

What Causes Resistance To Anthelmintics ???

- Frequent Treatments
 - ≥ 3 treatments per year
- Under dosing
- Using treatment strategies that minimize refugia
 - Treating all animals at same time
 - Treating when few larvae are on pasture
 - Treating early in grazing season
 - Treating and moving to clean pasture

Resistance Occurs Within Classes of Anthelmintics

- Resistance to one drug in a class confers resistance to all others (side resistance)
 - same mechanisms of action and resistance
- **Exceptions** to this are due to differences in potency and **are only temporary**
 - ABZ has better efficacy than FBZ
 - Levamisole has better efficacy than morantel
 - Moxidectin has better efficacy than IVM
 - Moxidectin kills ivermectin-R *Haemonchus*

Diagnosis of Anthelmintic Resistance



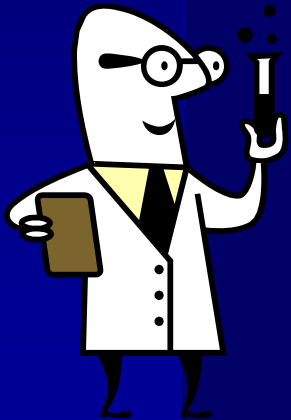
- In vivo tests are only real tools available for most hosts/parasites
 - Reduction in worm numbers – requires slaughter
 - Reduction in fecal egg counts (FECRT)
- FECRT - can be performed by a veterinarian in the field — simple anthelmintic trial
 - labor-intensive
 - high variability – but still fairly accurate
 - Most criticisms unfounded and based on having “a little knowledge”

Laboratory Diagnosis of Resistance



■ LDA - DrenchRite

- Only one test needed per farm
- One pooled fecal sample from 6 - 12 animals
- All 3 major drug classes tested in assay
 - Results for ivermectin used for moxidectin
- Interpretation of results is complicated with mixtures of species
- available as a diagnostic service in my lab
 - In past – discounted rate of \$175
 - As of 10/1/06 price = \$350



Fecal Egg Count Reduction Test (FECRT)



- Animals are divided into 2 or groups
 - Group 1 — Negative controls given nothing
 - Groups 2, 3, 4, etc — Test drug(s)
- Use large groups (10 - 15 animals)
- Groups should be balanced by sex and age
- Best to also balance by parasite load
 - Can use FEC or FAMACHA
- Should not have been treated for > 4 wks

FECRT

Sheep/Goats



- FEC 10 - 14 days after Tx
- Compare post-Tx mean of control group to means of drug - treated groups
 - $[(CTL\ FEC - Tx\ FEC) / CTL\ FEC] * 100$
- Analysis using RESO program
 - **effective anthelmintic** > 95% reduction in mean FEC compared to controls
 - **resistance** < 95% reduction AND LCI < 90%
 - **Equivocal (low resistance)**: 1 of 2 criteria met

RESO Program For Calculating Results of FECRT www.sheepwormcontrol.com

Detailed Calculations and Results

Combined Species

Drench	Pre-Test	Control	Levamisole	Albendazole	Ivermectin	Moxidectin	Drench 5	Drench
Number	10	10	10	10	10	10	10	0
Arith. Mean	1705	1830	15	845	1175	0		
Var (FEC)	2998583	4555111	1139	680806	1621250	0		
% Reduction			99	54	36	100		
Var (Reduction)			0.64	0.23	0.25	1.14		
Upper 95% CL			100	83	78	100		
Lower 95% CL			96	0	0	100		

Drench effectiveness		Susceptible	Resistant	Resistant	Susceptible
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Sp. Ostertagia:

Drench	Control	Levamisole	Albendazole	Ivermectin	Moxidectin	Drench 5	Drench
Number	10	10	10	10	10	10	0
% Total	0%	0%	0%	0%	0%	0%	0%

McMaster Fecal Egg Count

- Quick, easy to perform
- Should be part of routine parasite control program monitoring
- Easy means to determine efficacy of drugs
- Instructions and where to buy:
www.scsrpc.org



What is The Prevalence of Anthelmintic Resistance in Veterinary Helminths ???



Prevalence of Resistance on Goat Farms in Georgia (2001)

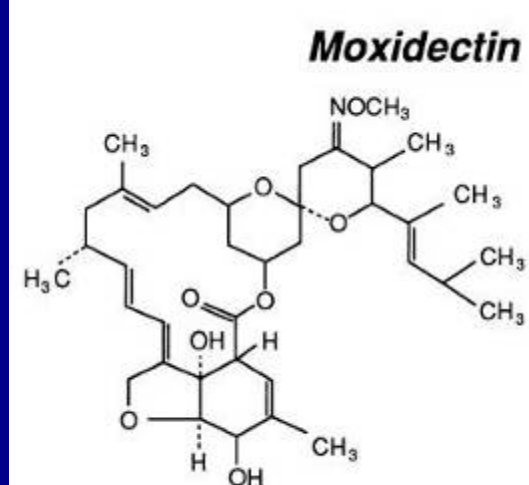
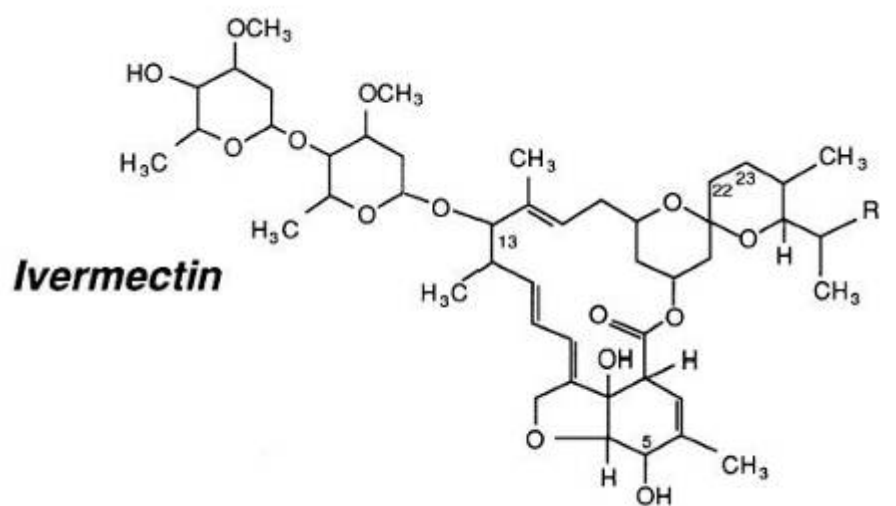
- Albendazole and Ivermectin
 - > 90%
- Levamisole
 - ~ 30%
- Moxidectin
 - None detected in 2001



Evaluation of prevalence and clinical implications of anthelmintic resistance in gastrointestinal nematodes of goats. Mortensen, et al., *JAVMA*, 223(4):495-500 (2003)

How Rapidly Will Resistance to MOX Develop Given a Background of IVM-R ???

- Evidence suggests that these drugs share the same mechanism of action
 - Resistance to IVM confers resistance to MOX
 - Potency issue -- similar resistance ratios



Objectives

On-Farm Trial 2003

1. Determine if resistance to moxidectin is developing on goat farms in Georgia
2. Examine the relationship between the *in vivo* FECRT and *in vitro* LDA
3. Establish reference values for Dx of moxidectin resistance using the DrenchRite[®] LDA

Methods

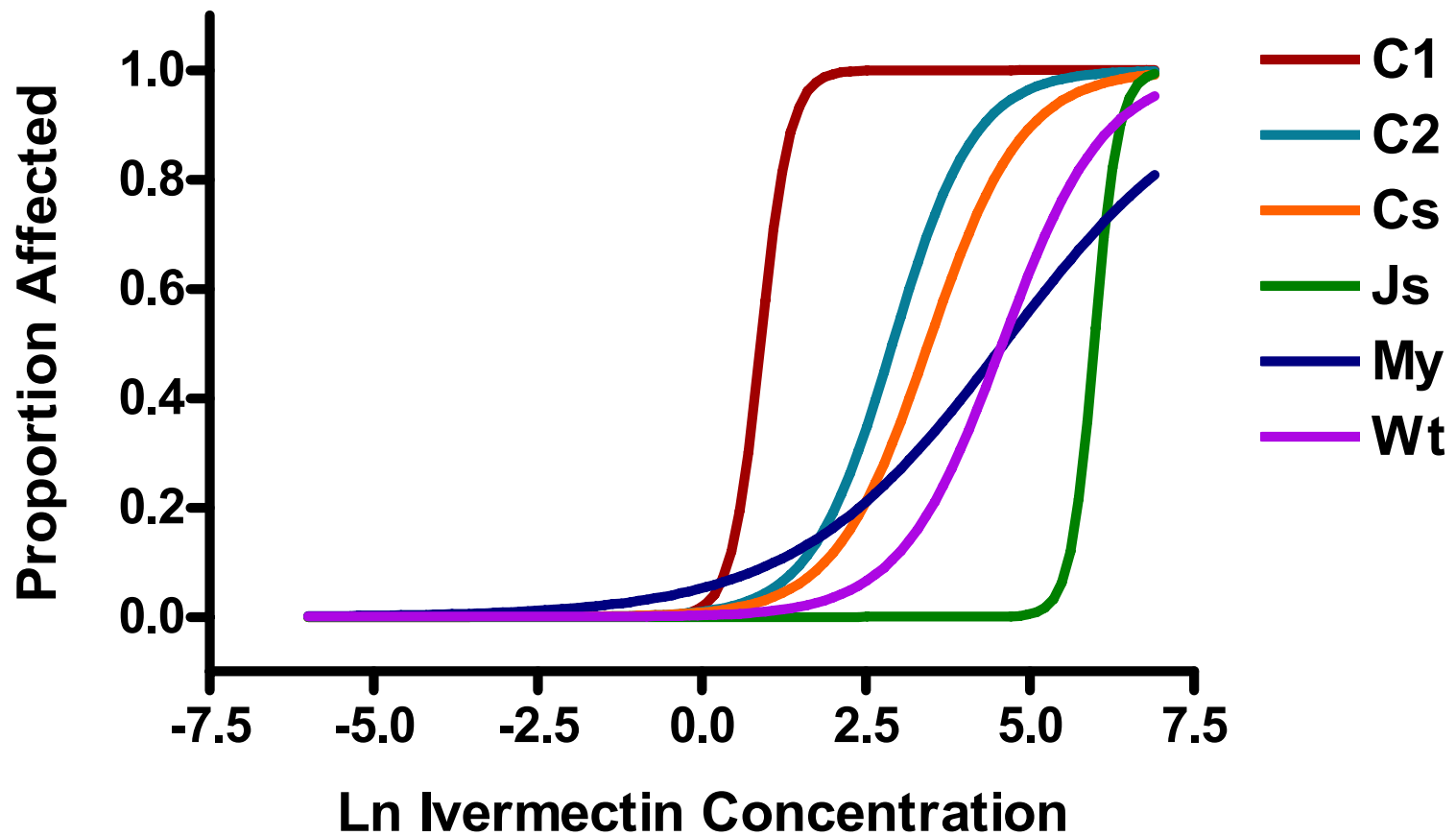
Fecal Egg Count Reduction Test DrenchRite[®] LDA

- No treatment control and moxidectin at 4 different dose levels
 - untreated
 - Mox 1 (10 or 25 ug/kg)
 - Mox 2 (25 or 50 ug/kg)
 - Mox 3 (100 ug/kg)
 - Mox 4 (400 ug/kg)
- DrenchRite LDA performed in duplicate for each farm



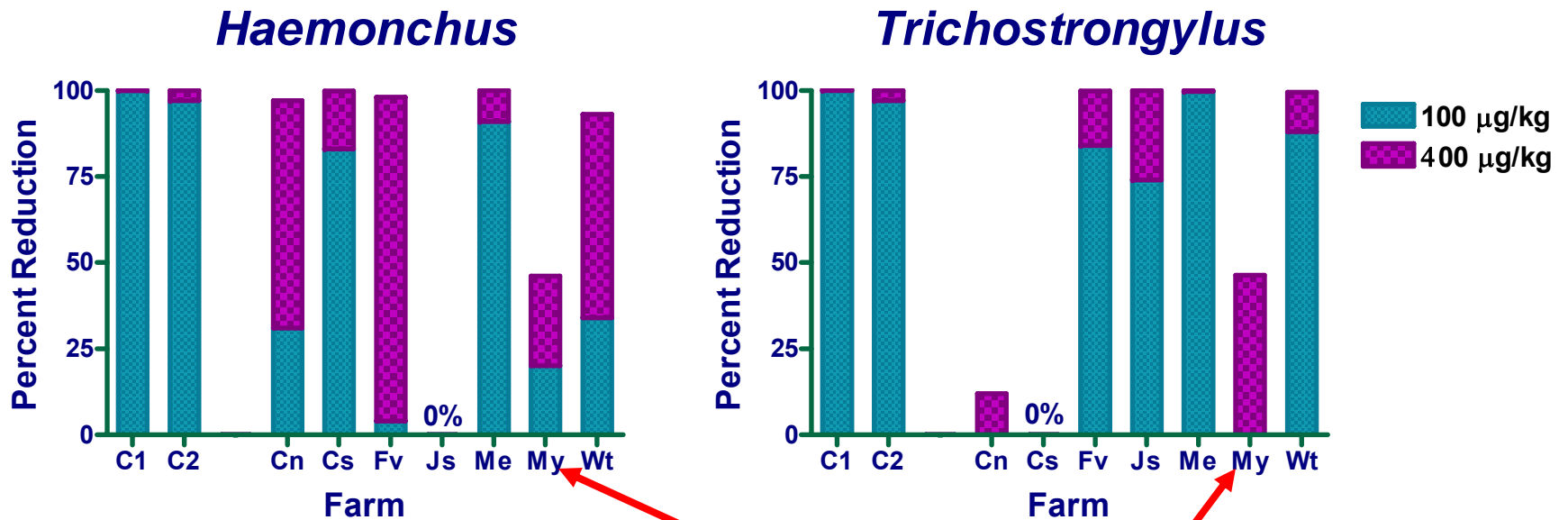
DrenchRite LDA

Dose-response for ivermectin/moxidectin



Fecal Egg Count Reduction Test

Moxidectin at 0.4 or 0.1 mg/kg



Both species resistant

What is the Prevalence of Moxidectin Resistance ???

- Unknown – but appears to be quite common
 - 2003 study: ~50% of farms with Hx of Cydectin use over previous 2 - 3 years had resistance
 - Resistance in both *Haemonchus* and *Trichostrongylus*
- It is now 3 years later
- Moxidectin must be used carefully and with prevention of resistance as a goal
 - Use only to treat severely clinically ill animals
 - Minimize use to preserve efficacy
 - Use injectable product in goats

Total Anthelmintic Failure

- A near-term possibility on many sheep and goat farms in many regions
 - Many farms are down to their last drug
- Malaysia and South America
 - Some farms reporting TOTAL drench failure
 - 20% death losses per year
- First case in USA diagnosed by my laboratory last year
- Future viability of small ruminant industries is threatened

Total Anthelmintic Failure on a Meat Goat Farm in Arkansas, USA

Haemonchus:

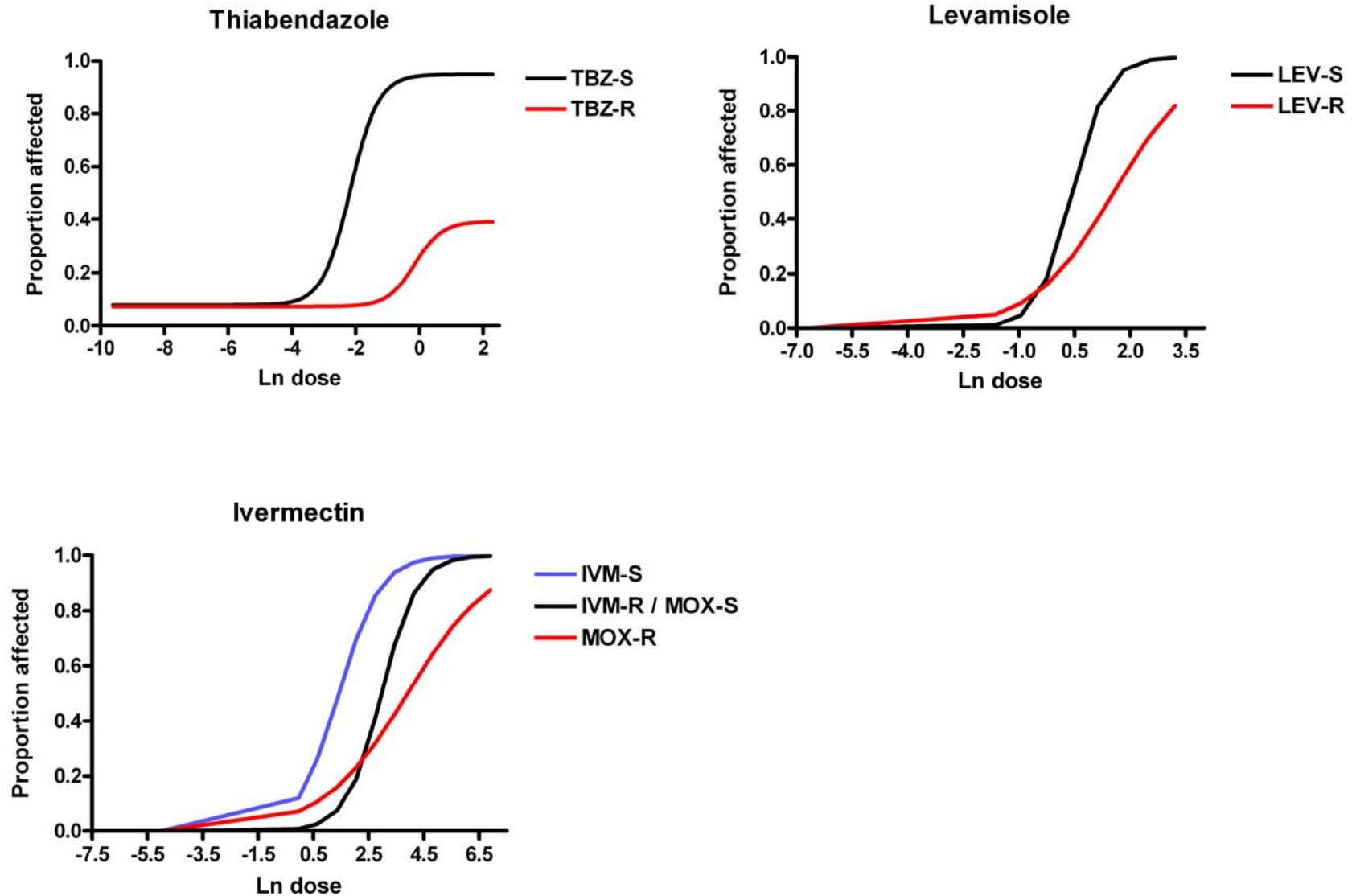
Drench	Control	Levamisole	Albendazole	Ivermectin	Moxidectin
Number	15	14	14	11	15
% Total	88%	71%	93%	96%	98%
Arith. Mean	2191	396	910	1937	552
% Reduction		82	58	12	75
Upper 95% CL		91	78	53	87
Lower 95% CL		65	21	0	52
Drench effectiveness		Resistant	Resistant	Resistant	Resistant

Trichostrongylus:

Drench	Control	Levamisole	Albendazole	Ivermectin	Moxidectin
Number	15	14	14	11	15
% Total	12%	29%	7%	4%	2%
Arith. Mean	299	162	69	81	11
% Reduction		46	77	73	96
Upper 95% CL		72	88	86	98
Lower 95% CL		0	57	49	93
Drench effectiveness		Resistant	Resistant	Resistant	Susceptible

Haemonchus contortus

Dose Response: Larval Development Assay



Prevalence of Anthelmintic Resistance in Sheep: New Zealand and Australia

- Australia (WA)
 - IVM – 60%
 - ABZ – 99%
 - LEV – 99%
- New Zealand
 - IVM – 25%
 - ABZ – 41%
 - LEV – 24%
 - Triple resistant – 7%

Moxidectin prevalence not determined but is becoming increasingly common

What Is The Prevalence of Resistance in Cyathostomes of Horses in the USA ???

- FECRT performed on 44 horse farms in the southern USA:
 - Pleasure horse, breeding and training
 - Georgia, South Carolina, Kentucky, Louisiana, Florida
- Large farms/stables
 - 24 or more horses
- Fenbendazole, Oxibendazole, Pyrantel Pamoate, Ivermectin



44 Farms - Southern US (2001)

Result	FBZ	OBZ	PP	IVM
Sensitive >90% reduction	0 (0.0%)	10 (23%)	19 (45%)	43 (100%)
Suspected-Resistant 80 - 90% reduction	0 (0.0%)	10 (23%)	6 (14%)	0 (0.0%)
Resistant <80% reduction	43 (100%)	23 (54%)	17 (41%)	0 (0.0%)

Prevalence of anthelmintic resistant cyathostomes on horse farms. Kaplan, et al., *JAVMA*, 225(6): 903-910 (2003)

Mean and (Range) Percent Reductions In FEC

State	FBZ	OBZ	PP	IVM
Georgia	27.4 ^a (0.0 – 71.8)	73.4 ^b (21.8 – 93.9)	82.7 ^c (6.6 – 100)	99.9 ^e (99.9 – 100)
Louisiana	34.6 ^a (6.3 – 77.5)	80.8 ^b (44.6 – 98.3)	86.3 ^c (63.7 – 99.0)	99.9 ^e (99.2 – 99.9)
Kentucky	18.2 ^a (0.0 – 81.3)	66.7 ^b (5.5 – 96.1)	65.1 ^d (4.5 – 99.8)	99.9 ^e (99.1 – 100)
Florida	19.9 ^a (0.0 – 72.7)	73.7 ^b (10.4 – 96.6)	78.5 ^{c,d} (31.8 – 99.9)	99.9 ^e (99.9 – 100)
4-State Mean	24.8	73.8	78.6	99.9

Ivermectin and Moxidectin Resistance in Cyathostomes ??

- Ivermectin resistance
 - “not a question of if, but rather when”
- First case reported at meeting of the WAAVP in 2005
 - Moxidectin resistance in cyathostomes infecting donkeys at the Donkey Sanctuary in the UK
- Is this an isolated occurrence or the tip of the iceberg ???

IVM Resistance in *P. equorum*



- Numerous reports of resistance to ivermectin and moxidectin in *P. equorum*
 - Based on poor levels of reduction in FEC
- Confirmed by my laboratory in 2005
 - Controlled efficacy study
 - Foals experimentally infected with an apparent resistant isolate from Canada
 - No significant difference in numbers of worms recovered from treated and control foals

Anthelmintic Resistance in Nematodes of Cattle

- Certainly less of a problem than with small ruminants or horses
- In Brazil, Argentina, and New Zealand
 - ML resistance is reaching levels of concern
- In other areas of the world too little data to know extent of problem
 - Numbers of reports increasing around the world
 - Problem may be more severe than is currently recognized

Anthelmintic Resistance in New Zealand Cattle

- Prevalence of anthelmintic resistance on stocker type cattle operations in NZ (as reported at 2005 WAAVP Conf)
 - IVM – 92%
 - ABZ – 76%
 - 74% resistant to both IVM and ABZ
 - Mostly *Cooperia*
 - LEV – 8%



Implications of Widespread Anthelmintic Resistance

- Management of anthelmintic resistance should be made part of all worm control practices and recommendations for horses, sheep, and goats
 - But is it really being done ???
- What about cattle ???

A Fresh Approach For Parasite Control Is Needed

- Frequent application of anthelmintics is no longer a viable approach
 - Non-medical, "recipe-based" approaches to parasite control cannot be advocated
- Effective anthelmintics must be thought of as extremely valuable and limited resources
 - A medically-based approach is required
 - "Intelligent Use of Anthelmintics"



Modern “Medically Based” Approach to Parasite Control

- Treatment decisions based on:
 - Biology of parasites
 - that are important – NOW
 - Resistance status of worms on a farm
 - Efficacy of drugs against particular parasites and stages of development
 - Dynamics of resistance selection
 - Biology of host – parasite relationship
 - Needs of individual patients
- Requires veterinary involvement
 - Significant diagnostic needs

Levels of Parasite Control Required Are Not the Same For All Animals

- Targeted Selective Treatment Approach
 - A new idea that has been around for a long time
 - Treat only those animals that require treatment
 - Parasites are highly overdispersed
 - 20 - 30 % of animals harbor most of the worms
 - System easily applied to horses and small ruminants

Thank You For Your Attention
Questions ???

