CHARLES STURT UNIVERSITY

AUSTRALIA

Anthelmintic Resistance – proposed measures to address it

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AUSTRALIA

Anthelmintic Resistance

Anthelmintics are agents used against helminth (worm) parasites

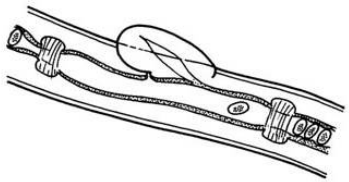
- How common is it?
- Which parasites, which drugs?
- What causes it?
- How do we deal with it?
  - Studies on resistance mechanisms
  - Prevention and control
  - New Chemicals
  - Registration process
  - Better diagnosis

Snapshot of Resistance

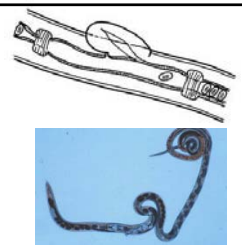
- PIGS
  - ***Oesophagostomum* spp.**
    pyrantel, ivermectin
    benzimidazoles
- HORSES
  - **Cyathostomins**
    benzimidazoles
    piperazine, pyrantel
  - ***Parascaris* ?**
    ivermectin
- HUMANS
  - ***Onchocerca volvulus*** *?*
  - ***Necator***
- DOGS
  - ***Ancylostoma*** *?*
- SHEEP
  - **Trichostrongyloids**
    benzimidazoles
    levamisole
    macrocyclic lactones
    closantel
- CATTLE
  - ***Cooperia* spp.**
    macrocyclic lactones
  - ***Ostertagia ostertagi***
    benzimidazoles
- RUMINANTS
  - ***Fasciola hepatica***
    triclabendazole

Resistance in scour worms
% of farms with <95% FECR

Drug	BZ	LEV	ML (IVM)	ALL 3	None
NZ *(Waghorn et al 2006)*	41	24	36 (mainly *Ostertagia*)	8	36
Spain *(Alvarez-Sanchez et al 2006)*	13	35	16	0	-



Resistance in Australia
% of farms with <95% FECR

Species	BZ	LEV	ML (IVM)	Closantel	None
	90	80	0-70	-	some
	>90	20	70	80	rare

Estimates from Love SJ NSW DPI

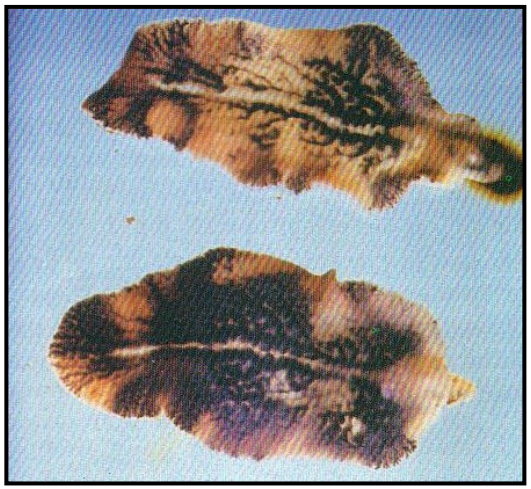
Resistance in Cattle

% of farms with <95% FECR

Species/ drug class	**BZ**	**LEV**	**ML** (IVM)
Cooperia	NZ 75% AU ~50%		AR 60% BR 92% NZ 90% AU ~ 69%
Haemonchus			BR 92%
Ostertagia	AR 32% NZ 26% AU ~50%	AU ~100%	NZ 8% AU ~9%
Trichostrongylus	AU ~50%		AU ~5%
Not identified	BR 20%	BR 8%	BR (MOX) 24%

US 2009: *Haemonchus* resistant to IVM and MOX
Cooperia resistant to IVM and MOX

NZ = Waghorn et al 2006 n=62
AR = Suarez and Cristel 2007 n=25
BR = Soutello et al 2007 n=25
AU = D Rendell, AVA Conf 2008 n=15
US = Garbarre et al 2009 n=1



Fasciola hepatica

Liver fluke in sheep and cattle

- Resistant to Triclabendazole
  - Sheep in Australia
  - Cattle in Australia?
  - Sheep and cattle in The Netherlands
  - Sheep and Cattle in Ireland

Horses

- Cyathostomins (small strongyles)
  - Benzimidazoles – 90% worldwide
  - Pyrantel – 20% - (not in Australia)
  - MLs – not in Australia
    - Reduced ERP (Germany)
    - ‘Resistance’ in donkeys (UK)
- *Parascaris*
  - Macrocyclic lactones (IVM, MOX)
    - International
    - Eggs persist after treatment (3 anecdotes from Australia)

What are the risk factors for resistance?

Generally: activities that allow resistance genes to be selected and reach the next worm generation

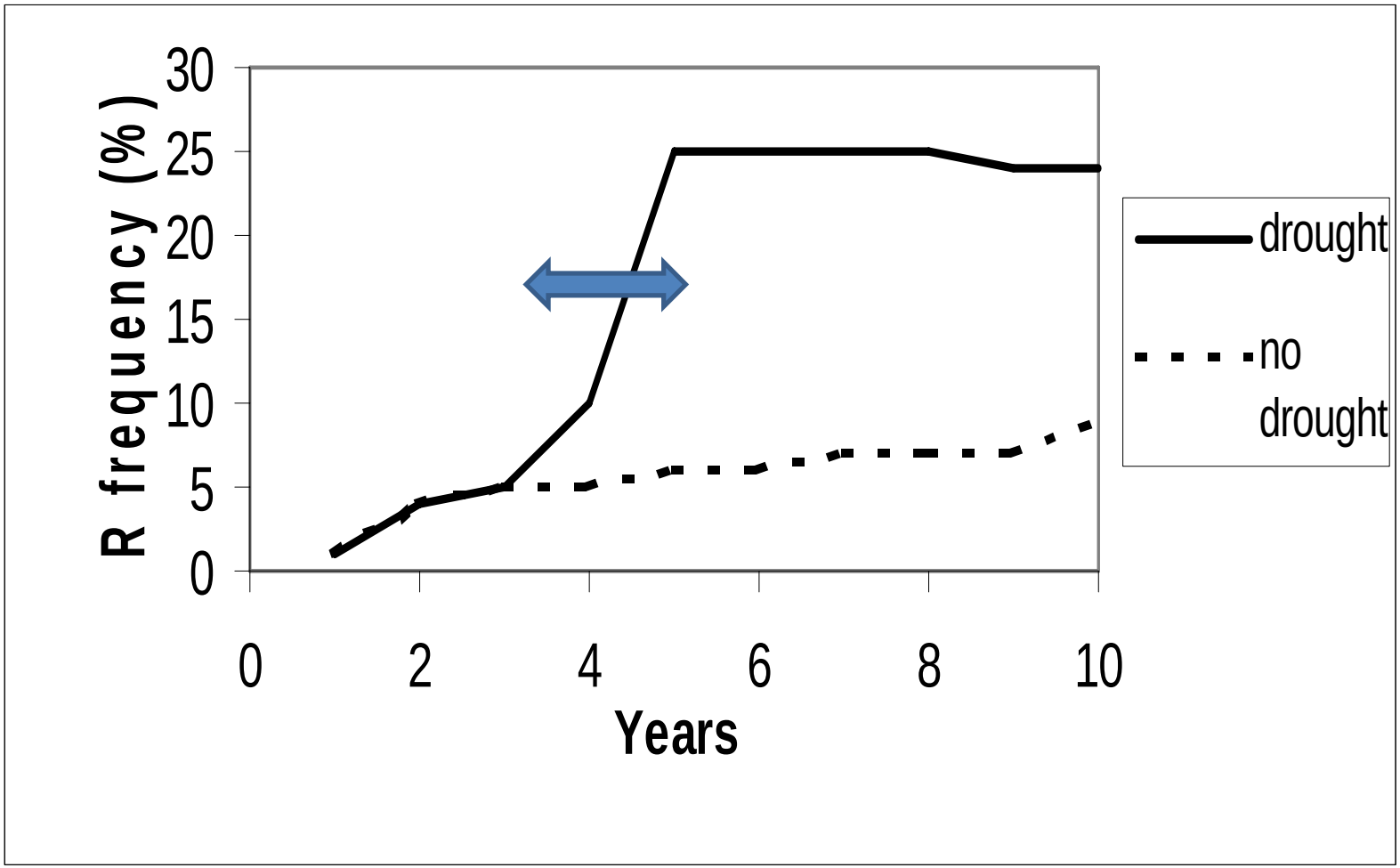
- Treatment of adult animals (eg. ewes near lambing)
- Treat and move to a clean pasture (also termed ‘low refugia management’)
- Lack of quarantine treatment
- Use of long acting agents including capsules
- Ineffective treatments and underdosing
- Excessive dosing

BUT management, environment and parasite biology influence the relative importance of these activities

Pomroy (2006) NZ Vet J 54;265

Climate simulation

Drought reduces refugia and increases resistance



Real world dilemma…

- Controlling worms with drugs risks developing resistance
- Not controlling worms with drugs = parasitism
- Controlling worms with prudent drug use and non-chemical methods is best practice

Best practice
Using IPM (monitoring, nutrition, sheep genetics, grazing management, targeted treatment)

Common practice
Little regard for prudent use

Resistance

- is inherited on chromosomal genes
  - No plasmids or other exchange of genetic material
  - Parasite species specific
- develops to each drug class independently
- resistance to one member of a class means there is resistance to all members of the class
  - Apparent susceptibility within a class is due to potency or pharmacokinetic differences

Resistance

- R genes are present at low levels in parasite populations
- You can select R with drug use
- Reversion to susceptibility is very slow
- R is not inevitable
- You can import resistant worms in animals
- R is not expressed to the same degree in all life cycles stages
  - some larval stages are useful for *in vitro* diagnosis

How do we deal with it?

- Studies on resistance mechanisms
- Prevention and control
- New chemicals
- Registration process
- Better diagnosis

Worm Genetics – the hope

- Find resistance alleles
- Understand selection
- Understand spread
- Inheritance
- Gene expression
- Genetic organisation
- Provide tools for diagnosis

Mechanisms of resistance

Drug efflux

Altered target

Drug metabolism

Drug permeability

- Affinity
- Physico-chemical
- Alternative pathway

Phenotype

Change in:

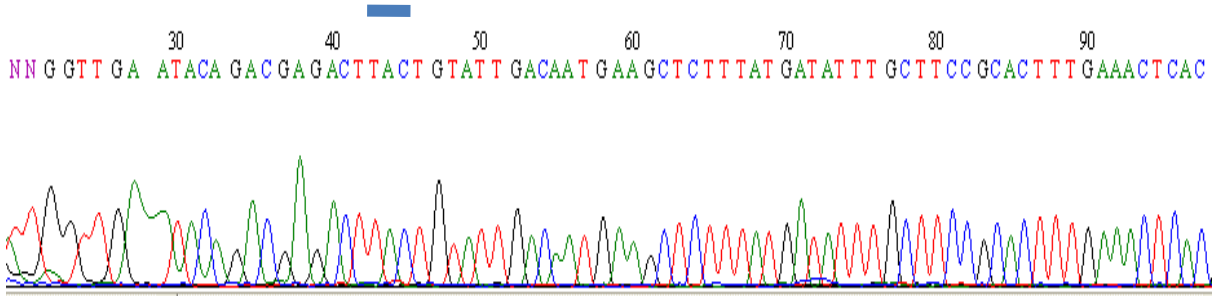
- AA sequence of target
- Structural protein
- Promoter
- Transposon silencing
- Expression pattern
- Upstream
- Downstream

Genetics of resistance

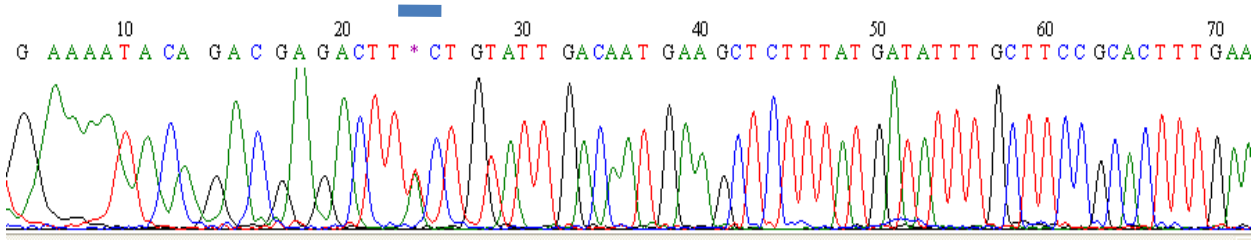
BZ resistance

- In several parasite species R is conferred by transition (SNP) in β-tubulin isotype 1
- TAC to TTC (Phe to Tyr) at:

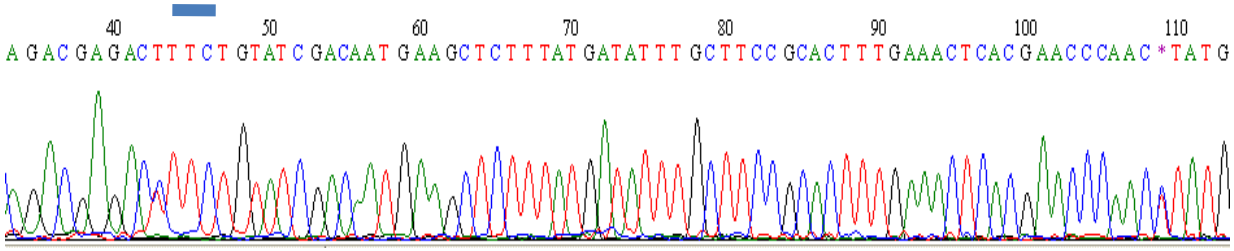
  F200Y, F167Y (and A198G) (assays)
- Phenotype associated with reduced drug affinity
- Function confirmed as R allele confers phenotype on *C. elegans* in heterologous expression
- F200Y has incomplete correlation with R phenotype (Other SNPs, isotype 2, Pgp, cytoP450)



Susc
TAC/TAC

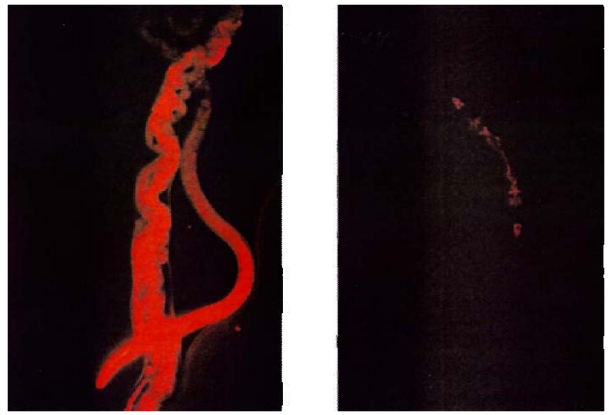


Heterozygote
TTC/TAC



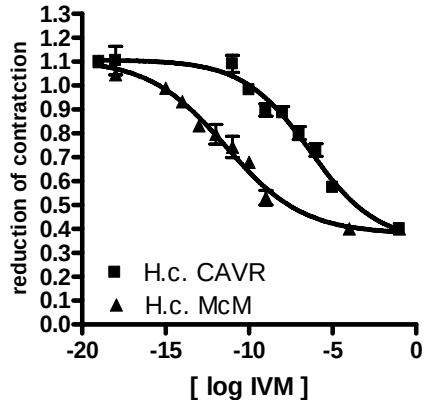
Res
TTC/TTC

IVM- resistance



Alleles:

- GABA receptor (ligand?)
- GluCl receptor (IVM receptor)
- Tubulin (BZ target)

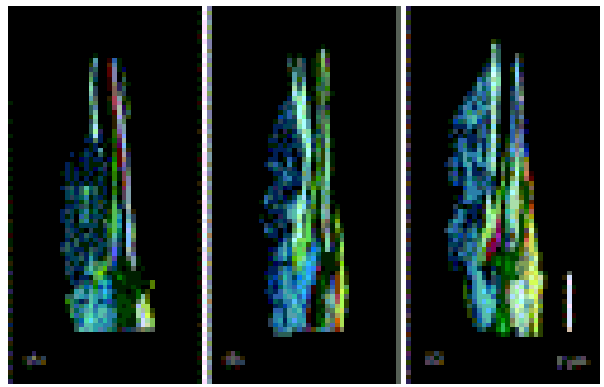


Transcription:

- Pgp (efflux)
- MRP (efflux)

Sites:

- Feeding (Receptor site)
- Body muscle contraction
- Amphids (permeability?)



Macrocyclic lactone resistance genes – *C. elegans*

GluCl α

Genes	Details	H.c. ortholog/	identity
avr-15	pharynx and motorneurones	none	--
avr-14 (*GluCl α 3A*)	nerve ring, motor neurons	*Hc gbr-2A*	88
(*GluCl α 3B*)		*Hc gbr-2B*	87
gcl-1	extrapharyngeal	none	--

Other genes:

osm- dyf- che-3 (dynein)	Dye filling	
unc-7 (innexin)	electrical leakage	
unc-9 (innexin)		
MDR, MRP	Drug efflux	Several
Thiol Metabolism	Drug efflux	Several

How to explain the riddles?

- Many genes responsible for resistance
- Looking in the wrong place
- Resistance phenotype
- Isolates, lab and field
- Genome stability
- Hitchhiking
- Genetic population structure
- Genetic diversity is huge

Mechanisms of resistance

Whole genome Approaches

Change in:
- AA sequence of target
- Structural protein
- Promoter
- Transposon silencing
- Expression pattern
- Upstream
- Downstream

- Affinity
- Physico-chemical
- Alternative pathway

Phenotype

Drug efflux

Altered target

Drug metabolism

Drug permeability

Genetics of resistance

Which approach?

Gilleard 2006 IJP 36, 1227

- ***candidate gene approach***

  Compare R and S using genes with presumed action and others that we can clone

- ***whole genome approach***

  Use molecular markers, DNA arrays, genome sequencing to compare S and R. No assumption about action.

Department of Agriculture

No.11/2005
Replaces No. 4/2002

Factsheet

Sheep worms — summer-autumn worm control

Rob Woodgate, Veterinary Officer, Albany and Brown Besier, Principal Veterinary Parasitologist, Albany

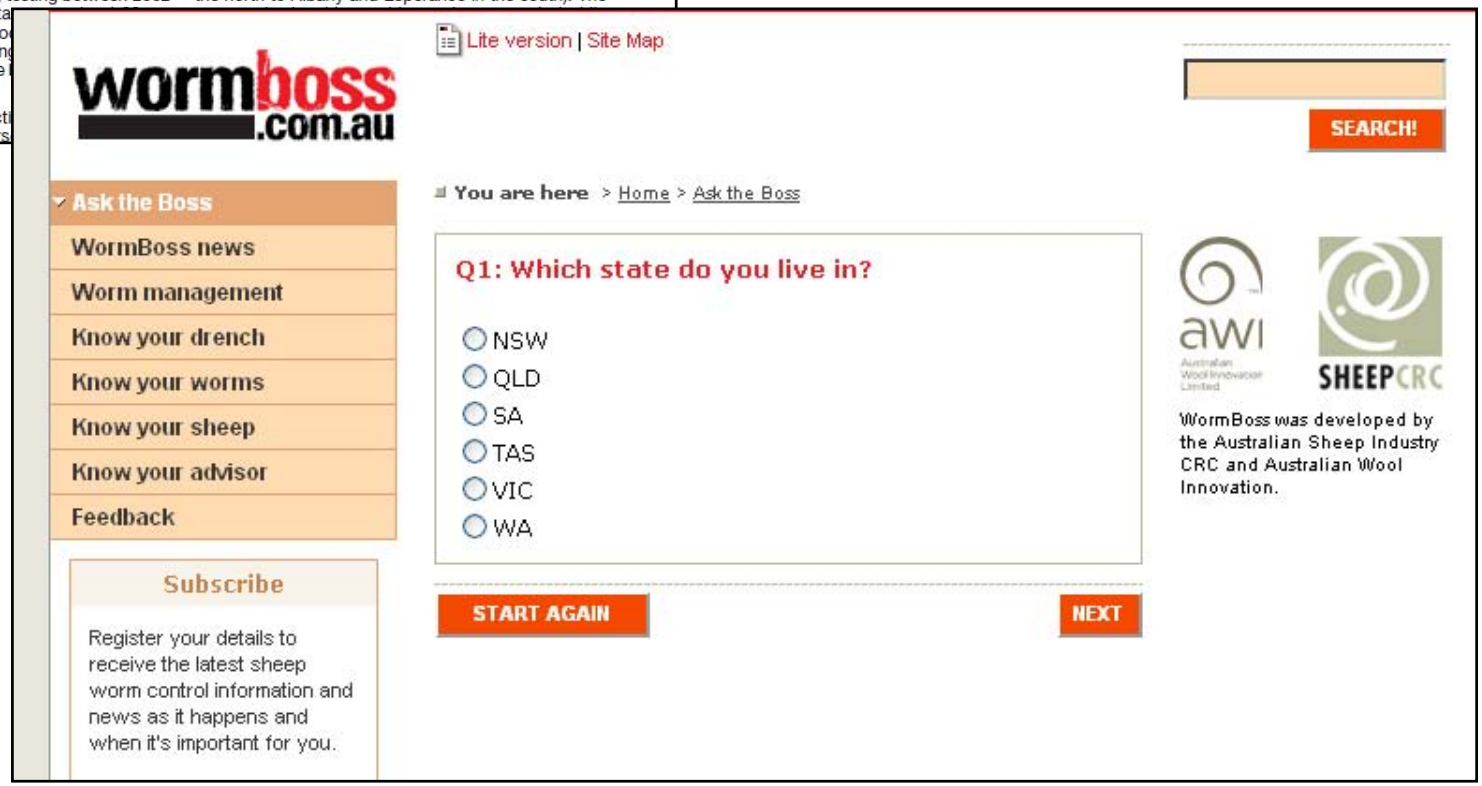
Background

Resistance by sheep worms to drenches in Western Australia is rapidly reaching a crisis point. There are worms resistant to white (benzimidazole or BZ – e.g. Valbazen, Panacur, Alben, Fenbendazole, Nemadet, Oxfen, Fencare, etc.) and clear (levamisole or LEV – e.g. Nilverm, Levamisole, Ripercol, etc.) drenches on virtually all farms. Worms on about 80 per cent of farms tested show resistance to BZ/LV combination drenches (containing a white and clear drench, e.g. Combi, Salvo, Scanda, etc.). Of most concern, testing between 2002 and March 2004 showed resista
cent of properties to the macro
of drenches (the MLs - active in
abamectin and moxidectin) in the
(*Ostertagia circumcincta*).

Unless current drenching practi
resistance will continue to wors

What is the answer?

Since 2001, Ag Department officers have monitored worm levels in weaners, ewes and dry sheep on more than 20 properties ('demo farms') throughout the State. This has included 6 key IPM-s sites (as part of the national Integrated Parasite Management - sheep project funded by Australian Wool Innovation Ltd) since 2004. The work involves collecting on-farm worm and management data at locations covering all major sheep producing regions (from Northampton and Moora in the north to Albany and Esperance in the south). The



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Q1: Which state do you live in?

- NSW
- QLD
- SA
- TAS
- VIC
- WA

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WormBoss was developed by the Australian Sheep Industry CRC and Australian Wool Innovation.

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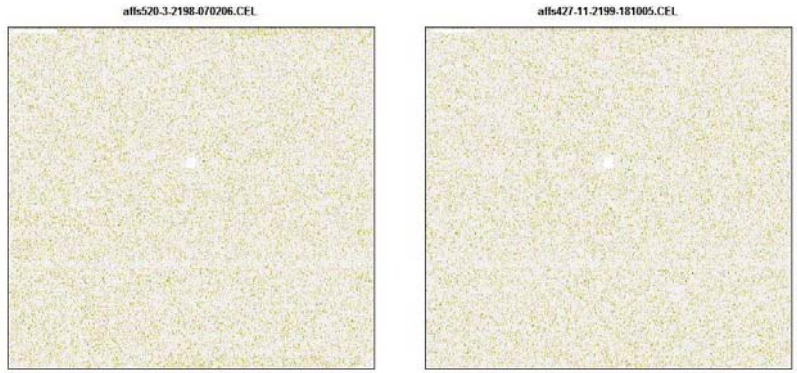
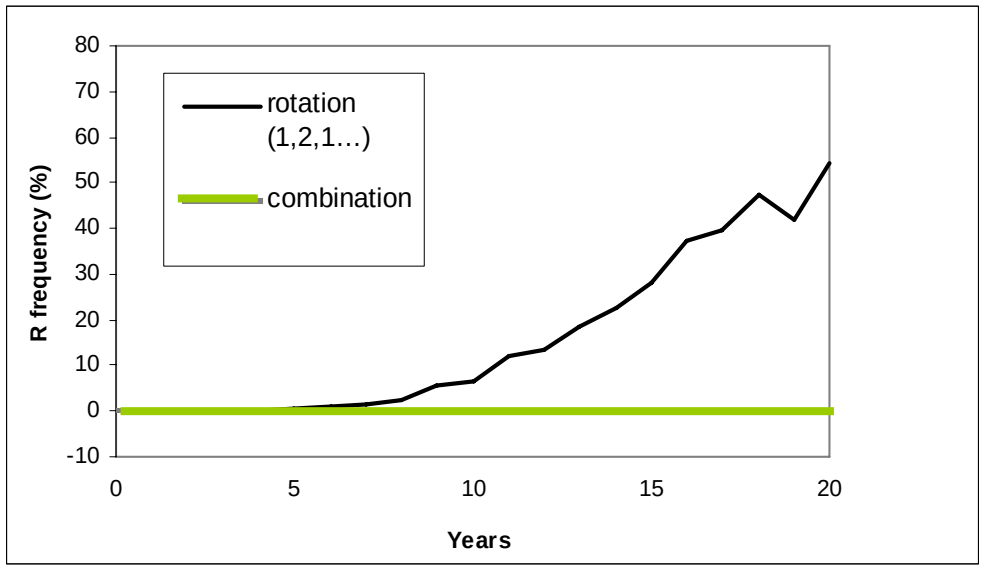
Register your details to receive the latest sheep worm control information and news as it happens and when it's important for you.

Prevention, and control

Control options

- Host genetics – breed for resistance
- Grazing tools
  - Fungi
  - Dung removal
- Vaccines
- Combinations





Novel anthelmintic chemicals

Derquantel + Abamectin (Startect®)

- Spiroindole (SI) + ML
- Derquantel acts as a selective cholinergic antagonist in somatic muscle
- Flaccid paralysis of treated worms
- Effective against parasites resistant to all current anthelmintics

Monepantel (Zolvix®)

- Amino acetonitrile derivative
- Acts at a nematode-specific acetylcholine receptor on muscle
- Hypercontraction of body and spasmodic contraction of pharynx
- Effective against parasites resistant to all current anthelmintics

Registration of chemicals against resistant isolates

- Guidelines
  - Moving targets?
  - Review ‘old’ products.
- Isolates for use in trials
- Field sites
- Overseas data

Isolates and strains

VICH GL 7 ANTHELMINTICS
DEFINITIONS:
FIELD ISOLATE: A collection of a sub-population of helminths for the conduct of drug effectiveness tests isolated from the field less than 10 years ago. The helminths are considered representative of current parasitic infections in the field and have been characterized (source, date, location, previous anthelmintic exposure and maintenance procedures)

LABORATORY STRAIN: A sub-population of helminths that has been isolated from the field at least 10 years ago, characterized and has been segregated in the laboratory based on a particular property which makes it unique for research areas such as resistance to certain antiparasitic compounds.

Sheep, cattle, horses?

Testing for efficacy

- Pen trials (characterised isolates)
- FECRT in the field (larval differentials, confirm with worm counts)
- Lack of definition of isolates (species, source, history, passages)
- Maintained in hosts (expense)
- No good genetic markers for confirmation

Better diagnosis

- Of parasitism

- Of resistance

Test pros & cons

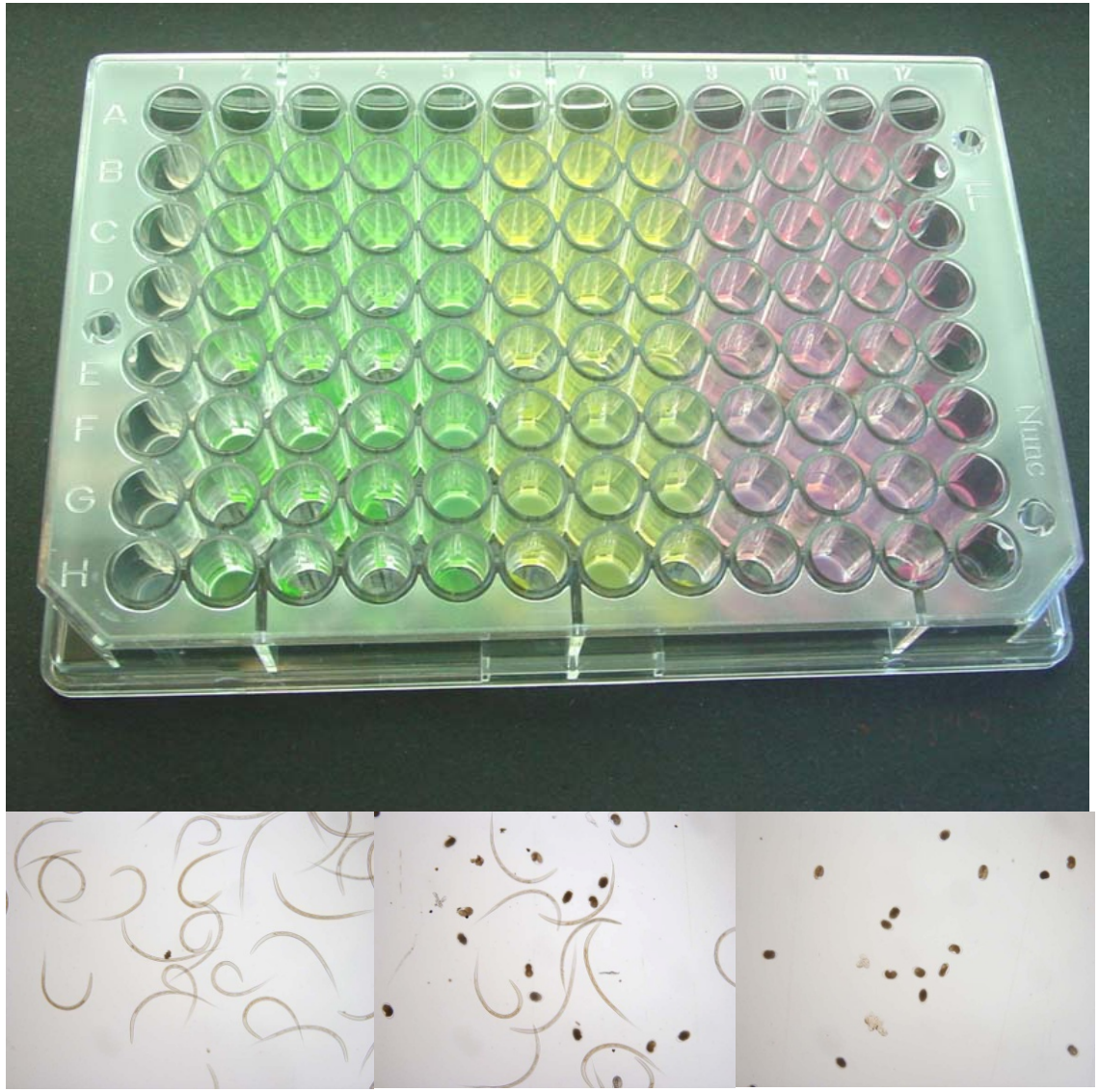
Test type	Pros	Cons
Worm counts	Direct answer. Species ID is easy.	Dead animal, ethics. Infrastructure needs.
Egg counts	May correlate with wc. Widely used. Sampling simple.	Poor estimate for adults in cattle, fluke infection, *Parascaris* etc. Variation
Larval differential	Add value to egg counts.	Statistical errors are compounded. 1 week delay. Higher skill.
Immunologically based	ELISA platform. Quantitative and HTP. Potential as herd test.	Antigen- eg .faecal, HWD Antibody – memory. Potential lack of specificity.
Molecular	Potential for high specificity and sensitivity.	Sampling and cleanup problematic. No ‘cow-side’ quantitative technology.

Better FECRT

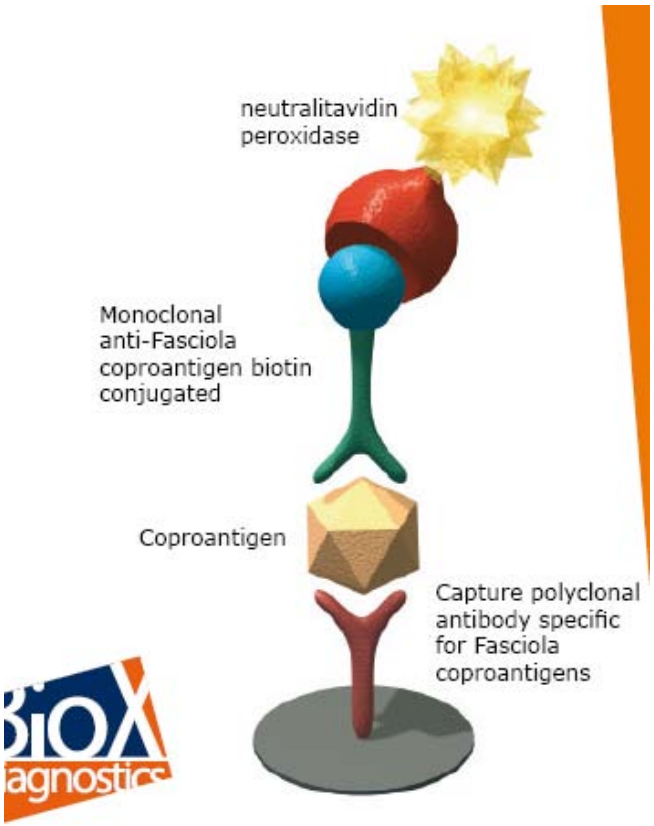
- New protocols
- Individual animal as own control
- More sensitive egg counts
- Statistics designed to minimise false detection rate

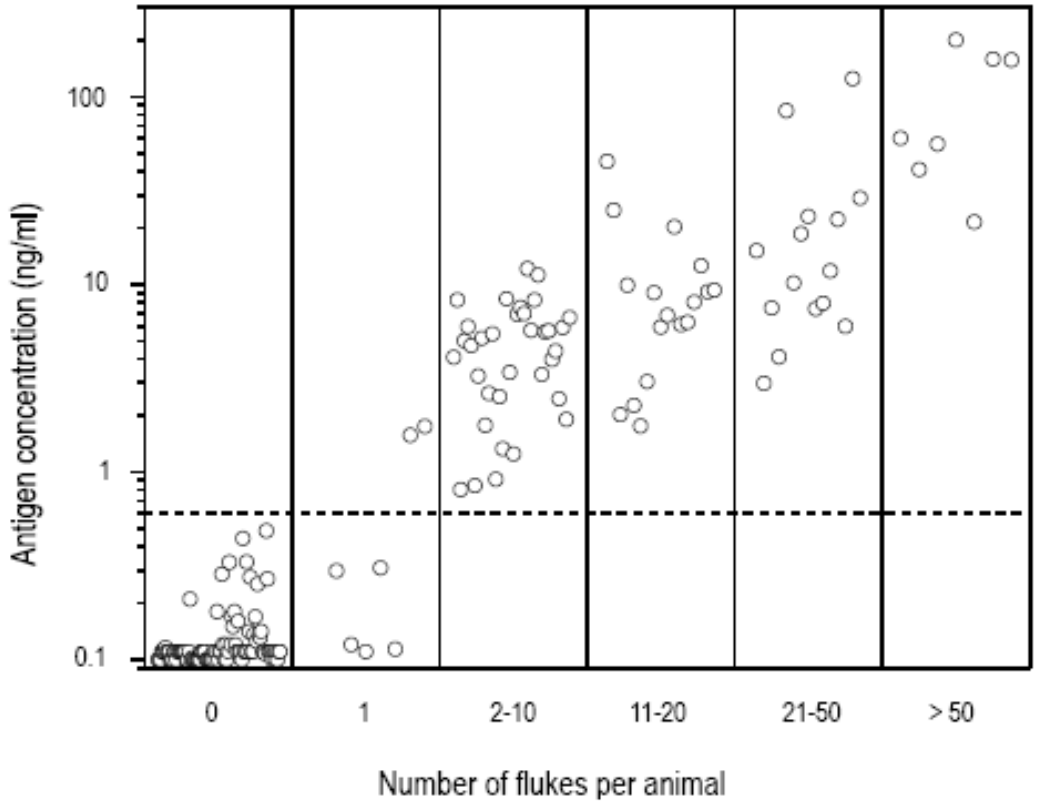
Drenchrite

- different drugs down the plate and increasing concentrations across
- Eggs cultured to larvae on plates
- Eggs and larvae are counted



Fasciola faecal antigen





Fasciola hepatica coproantigen concentrations measured by ELISA in fecal supernatants from 180 cows killed at the slaughterhouse. The dashed line is the detection limit in the ELISA assay (0.6 ng/ml, corresponding to the lowest concentration giving an OD reading 0.15 higher than the cutoff. (Mezo et al. 2004).

I think the worms are still winning!