Sustainable Control of Parasites in Sheep

PROCEEDINGS OF A WORKSHOP TO DISCUSS LIVER FLUKE CONTROL IN CATTLE AND SHEEP

8th -9th August 2012

Introduction

2012 was one of the wettest summers on record on many farms in the UK, providing the perfect conditions for the early stages of the liver fluke (Fasciola hepatica) and it's intermediate host the mud snail (*Galba*). This has resulted in a very high risk of liver fluke disease for cattle and sheep farmers not only in areas where we traditionally expect to see a significant challenge, but also in those areas not normally associated with liver fluke. Such have been the extremes that even on individual farms, the risks have shifted. For example:

- where flooding has washed the mud snails into areas on the edge of flood plains,
- poaching by animals has created new snail habitats
- changed grazing
- large numbers of unfinished lambs left on farms

These factors have combined to produce a huge threat to sheep and cattle farmers over the coming autumn and winter months. However, the risk will vary from area to area and farm to farm so a risk assessment and knowledge of the individual farm is essential, if management and treatment options are to be chosen correctly. Clear advice on the tools available for assessing risk and the management and treatment options available are urgently needed. This is particularly important as there are increasing reports of resistance to Triclabendazole (TCBZ), which is the only chemical available that will kill immature fluke, and so our ability to control the acute form of disease is largely reliant on retaining the efficacy of this treatment.

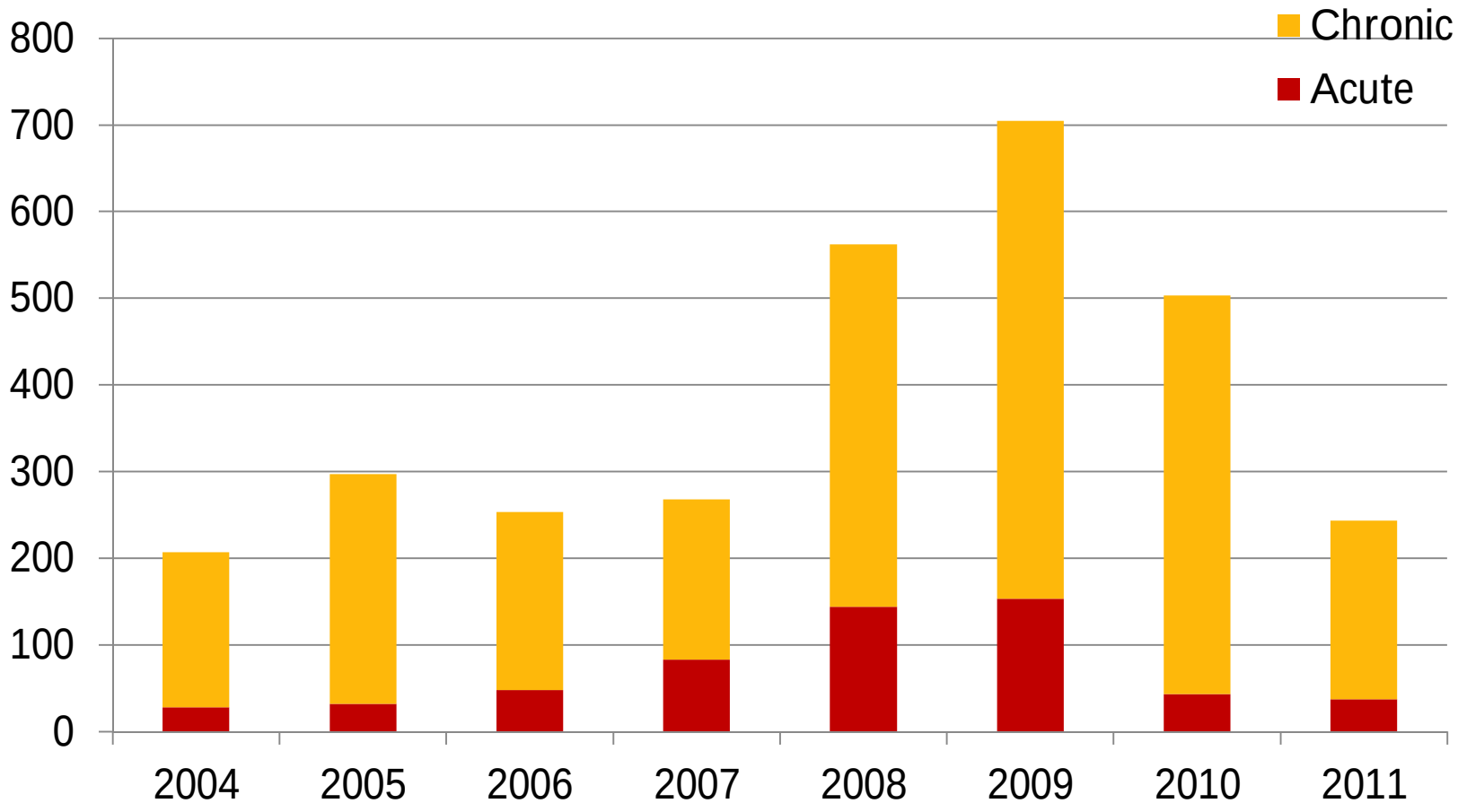
SCOPS convened a workshop of experts in August 2012 to review the situation, discuss the tools available to the UK sheep and cattle industry and provide the best possible advice for the challenge ahead. The group consisted of parasitologists, practicing Vets and those involved in field investigations together with farmer and adviser representatives. These Proceedings contain the presentations given by the experts and additional points made in discussion to help Vets and advisers.

The summary of the advice given to the industry is also included in the Appendices and more details can be found in Chapter 7 of the SCOPS Technical manual our website www.scops.org.uk/vets-manual.html

SCOPS would also like to thanks Novartis Animal Health, without whose financial assistance this workshop would not have been possible.

LIVER FLUKE
Introduction

Reported Liver Fluke in Sheep



Veterinary Investigation Diagnosis Analysis (VIDA) figures of acute and chronic fasciolosis in sheep

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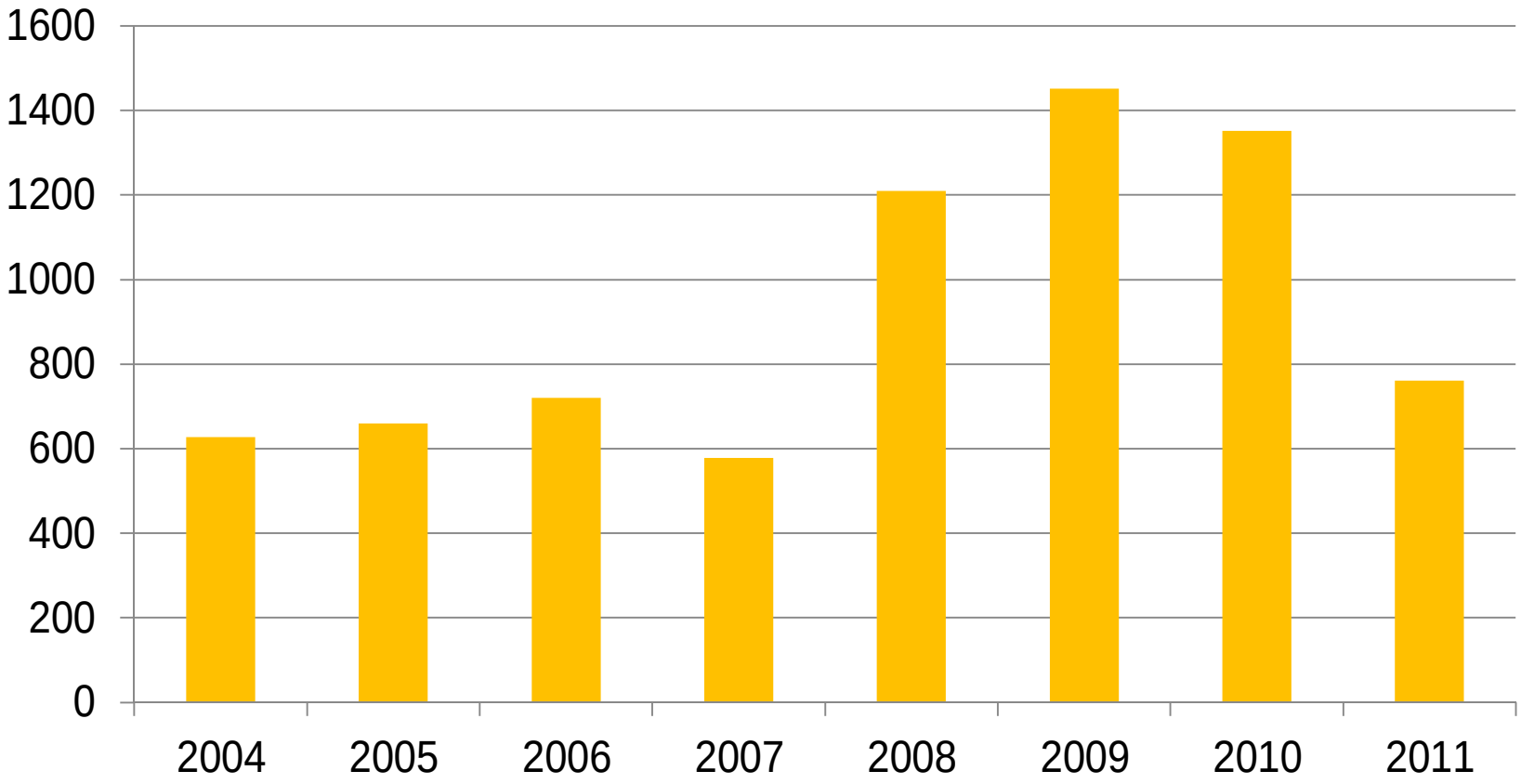
Reported Liver Fluke in Cattle



Veterinary Investigation Diagnosis Analysis (VIDA) figures of fasciolosis in cattle

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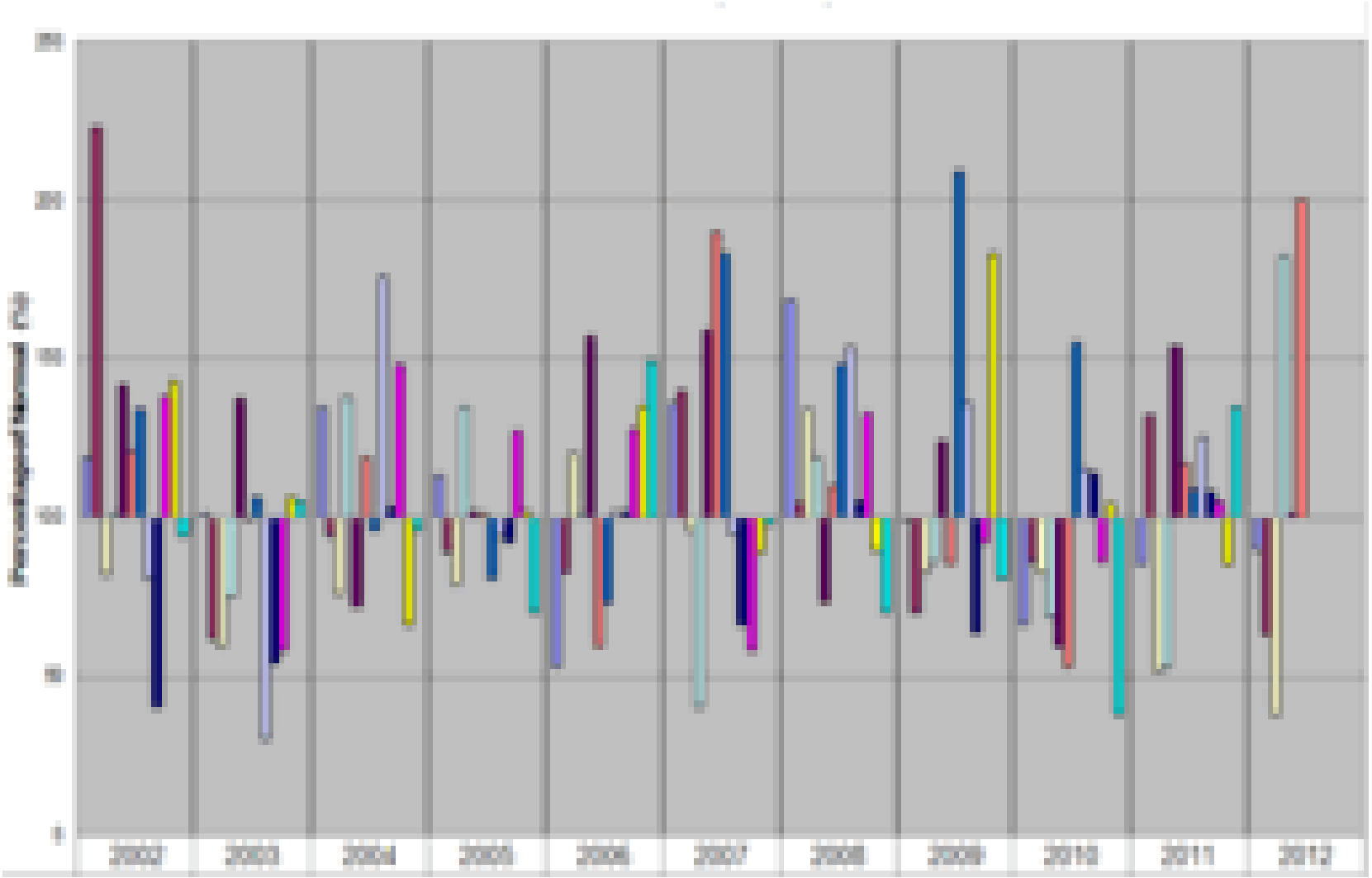
Rainfall Anomalies UK (2002-2012)



LIVER FLUKE
Forecasting Risks

Mike Taylor
VParST Ltd

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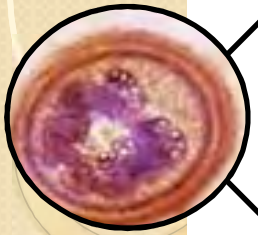
LIVER FLUKE



Caused by the trematode parasite, *Fasciola hepatica*

Digenetic trematode with snail intermediate host, (*Galba*) and several free-living stages

Incidence of liver fluke is far greater in areas with high summer rainfall

Hatching of fluke eggs and snail multiplication depends on adequate moisture and temperatures >10ºC

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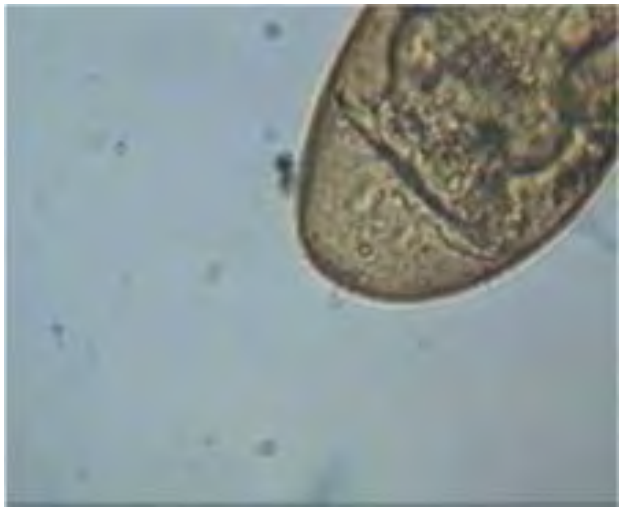
FLUKE EPIDEMIOLOGY

The main factors influencing the epidemiology of fluke infections are:

- **Rainfall**
  - Increases availability of snail habitats
  - Provides optimal field conditions for:
    - Development of fluke eggs
    - Miracidia searching for snails
    - Development of intermediate fluke stages within the snail
    - Dispersal of cercariae from snails



- **Temperature**
  - Hatching of fluke eggs and multiplication of snails depend on temperatures >10ºC
  - Temperatures >15ºC lead to significant multiplication of snails and fluke stages

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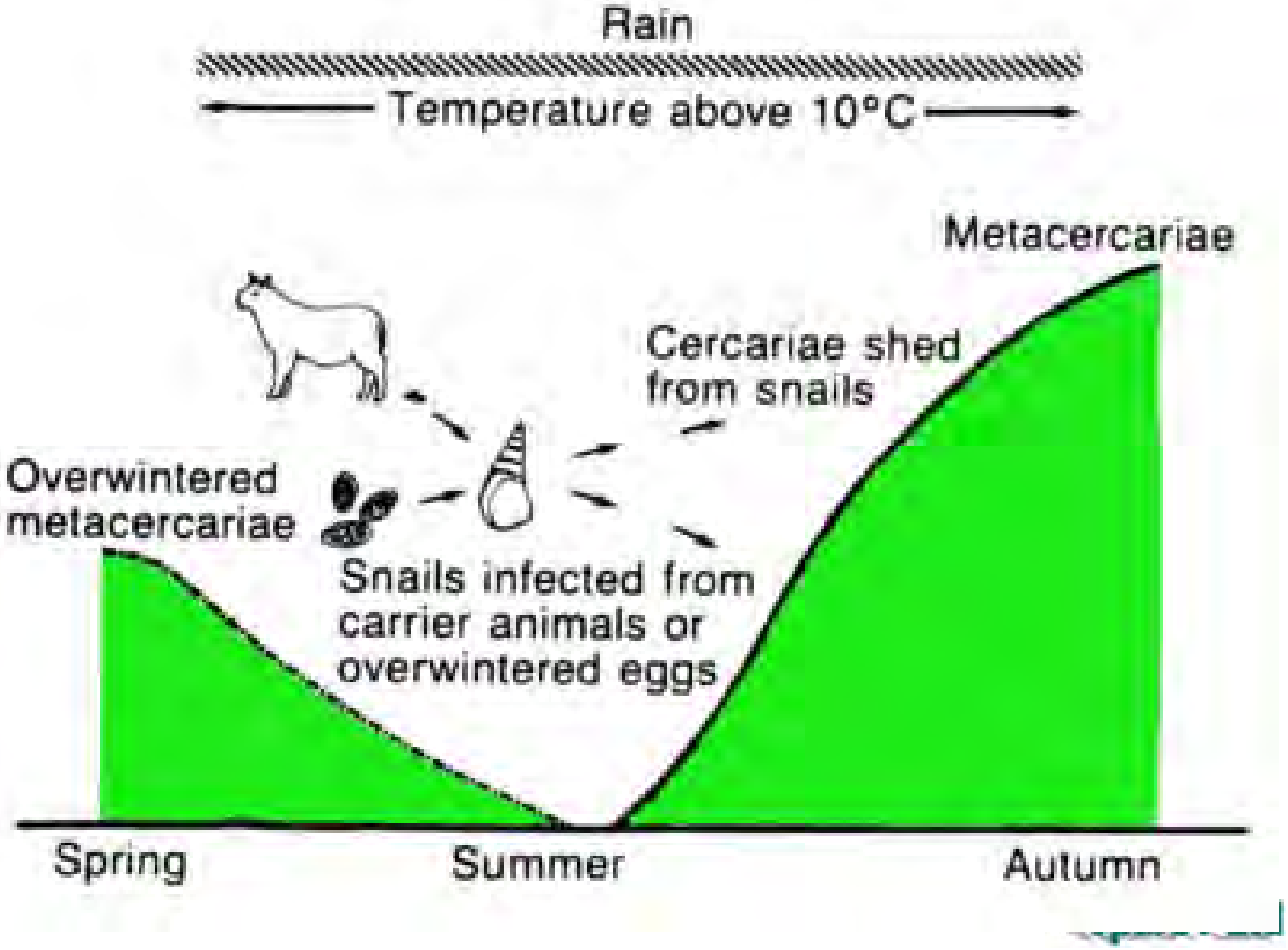
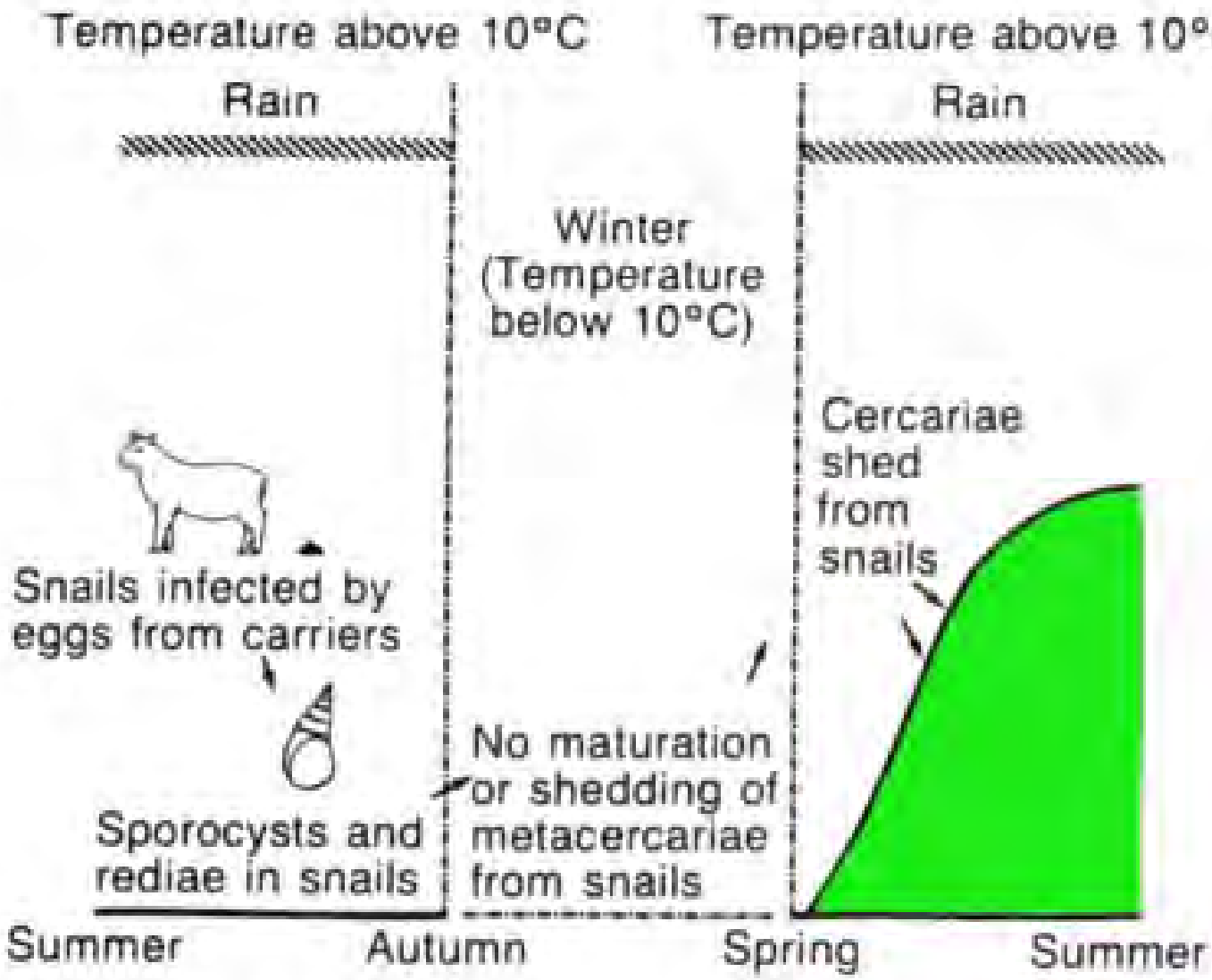
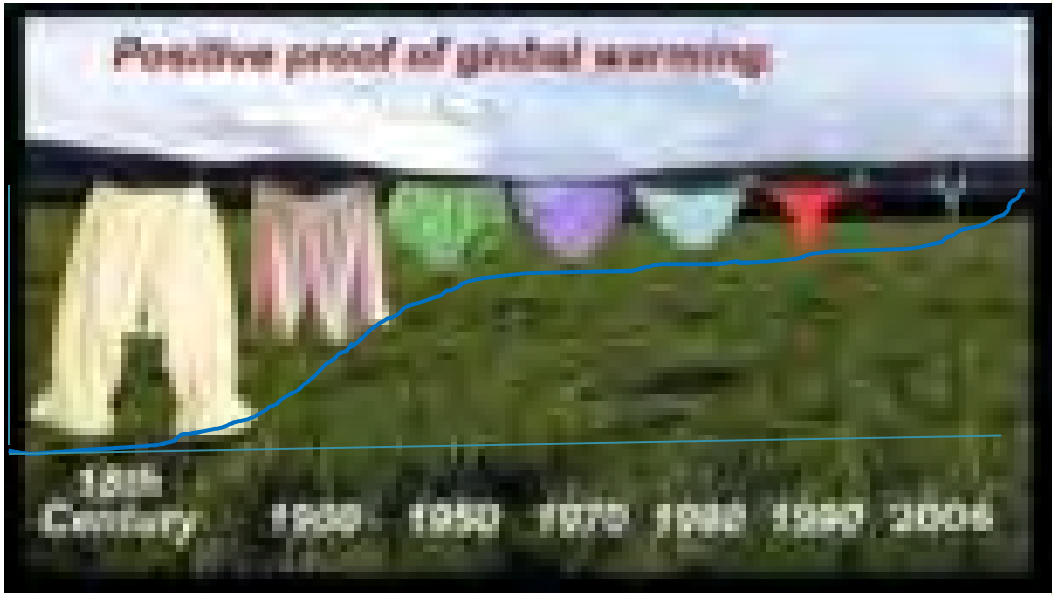
Summer Infection of Snails



Winter Infection of Snails



CLIMATE CHANGE?



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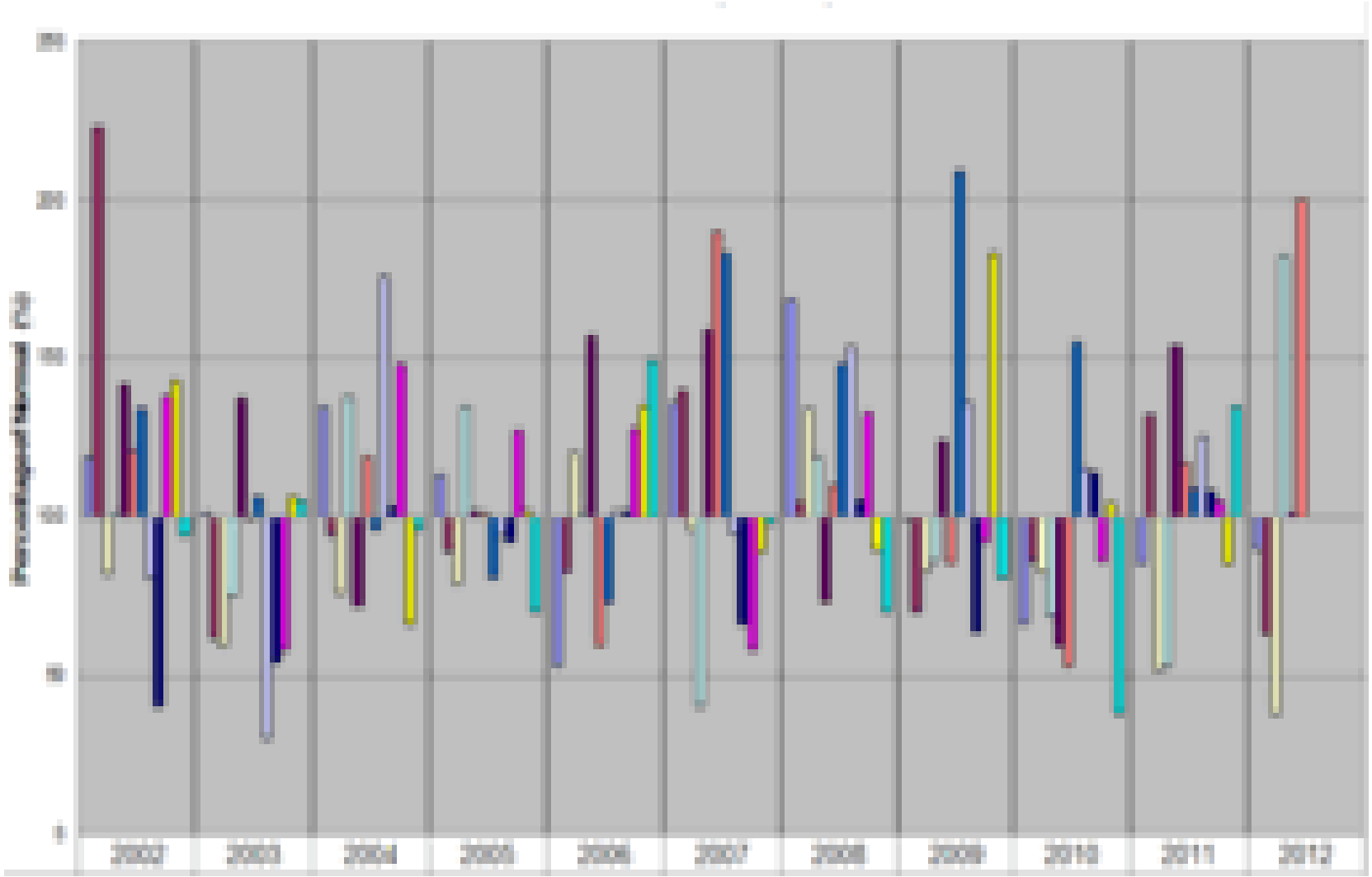
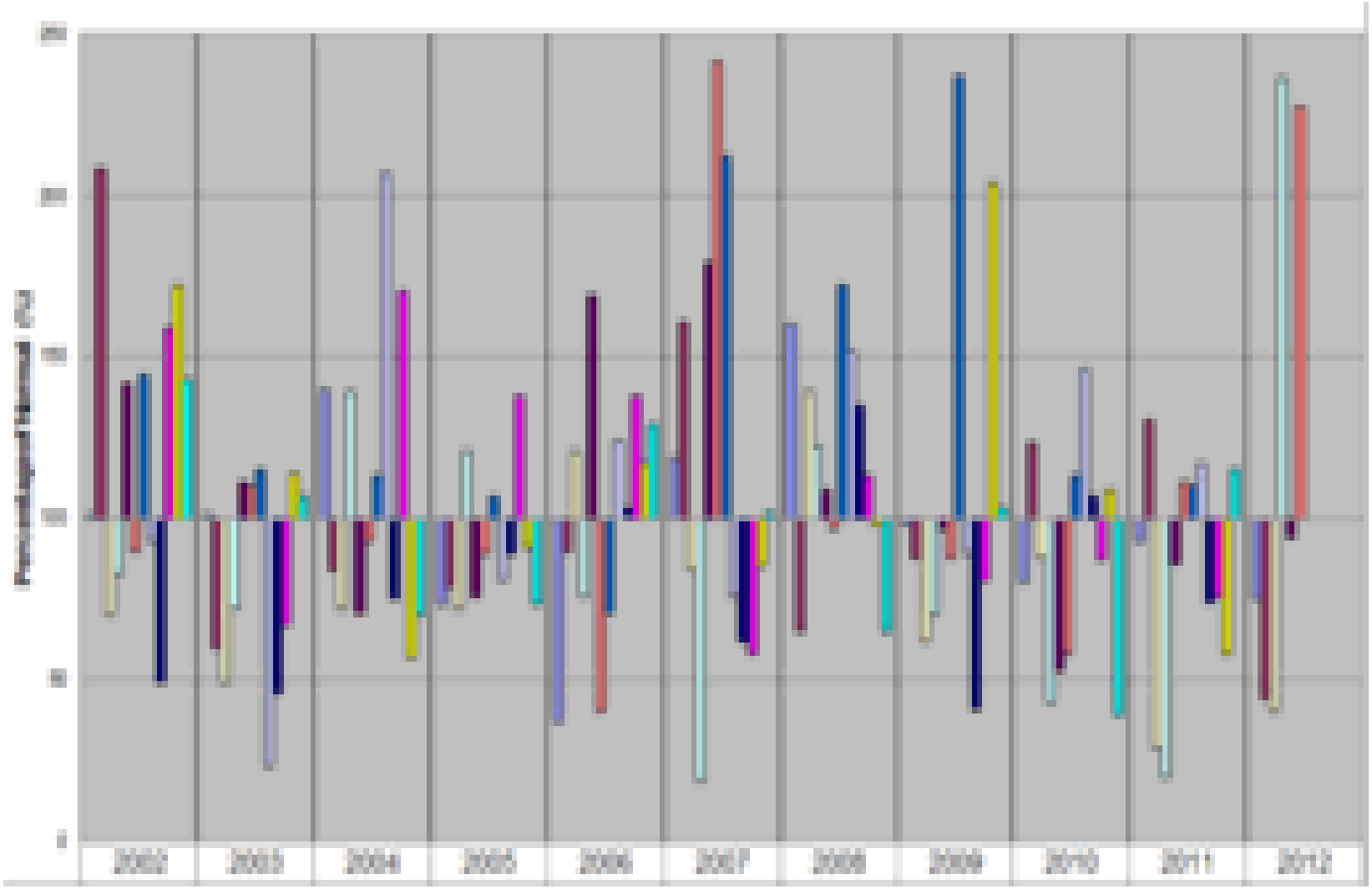
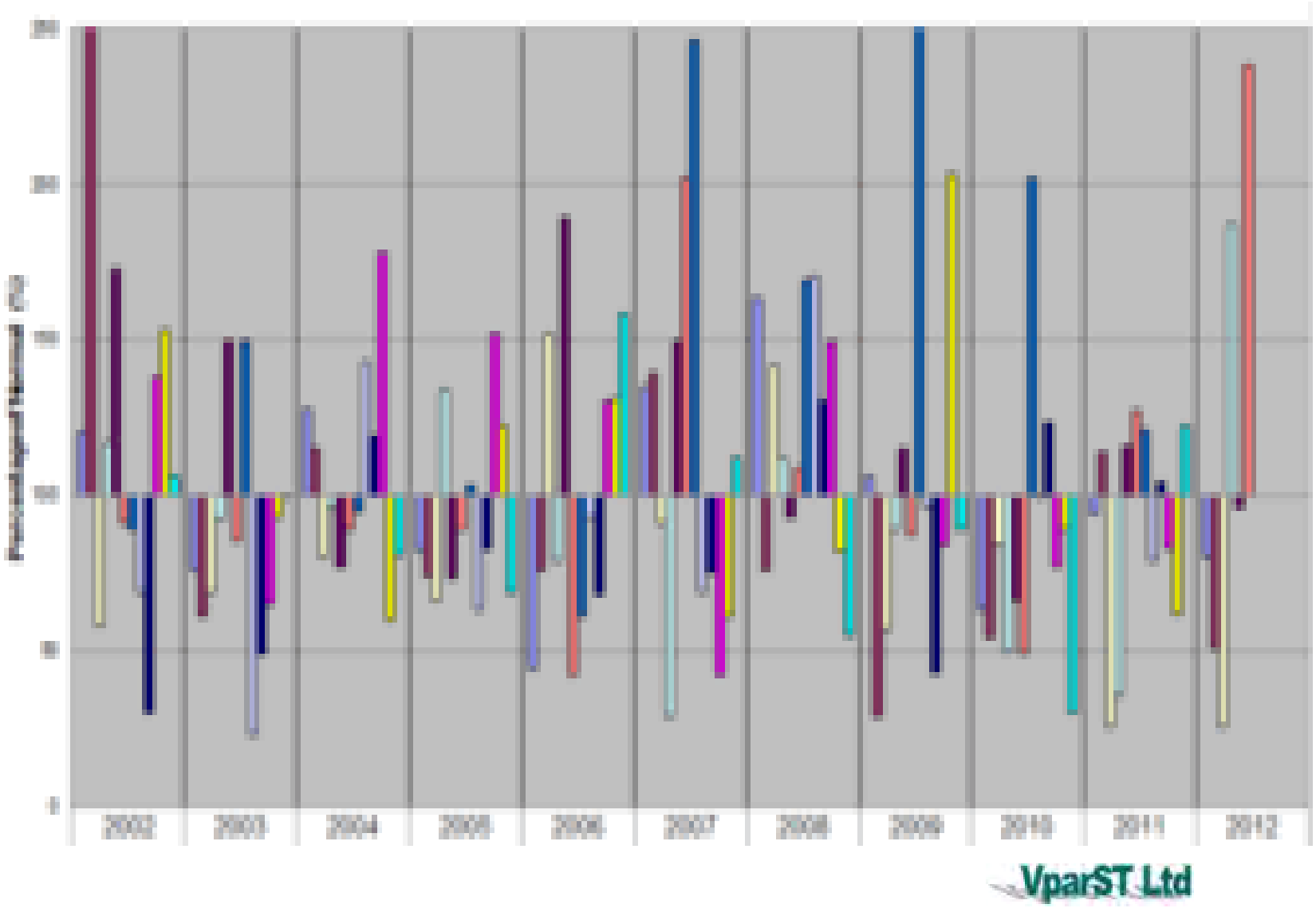
Rainfall Anomalies UK (2002-2012)



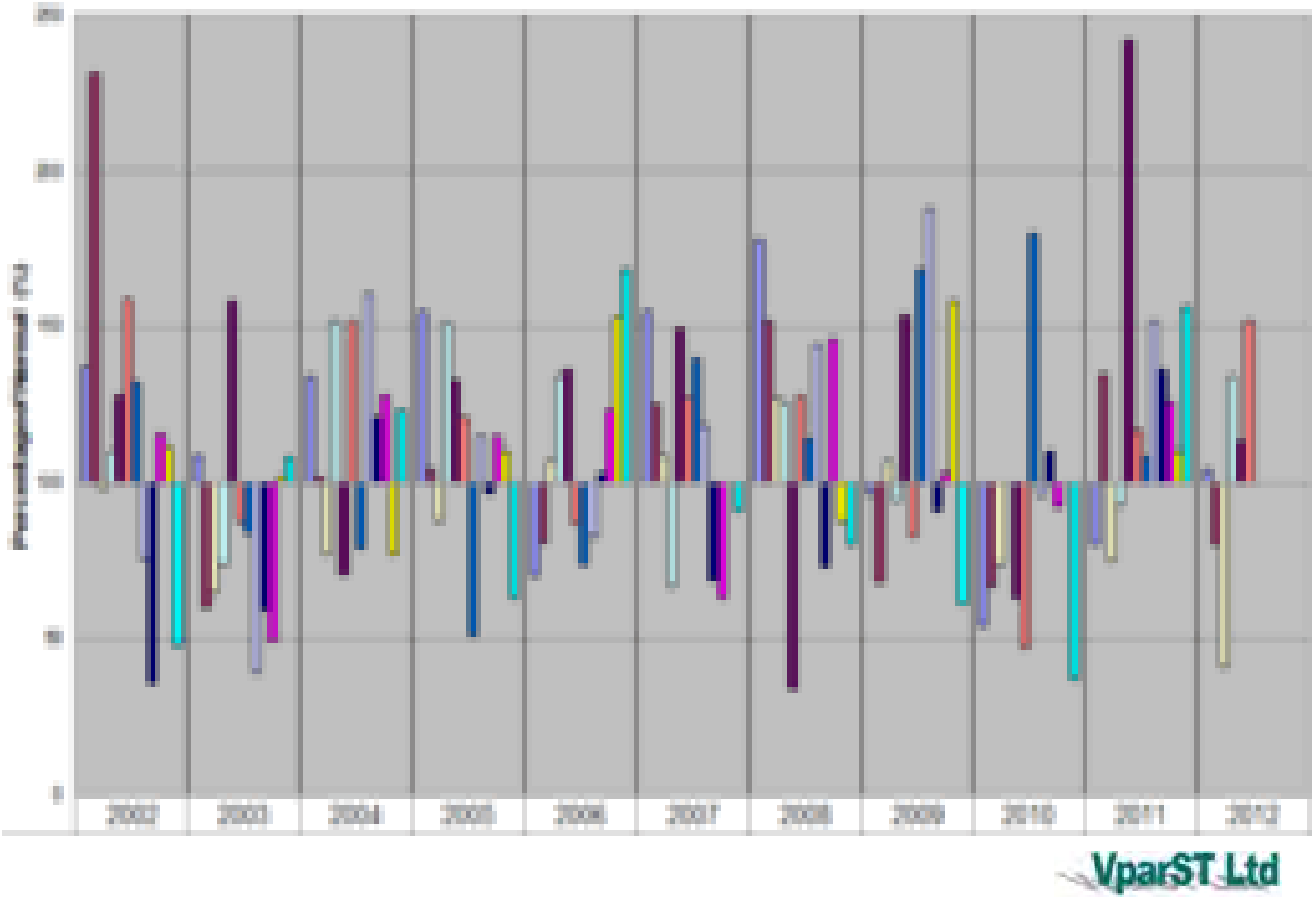
Rainfall Anomalies England (2002-2012)

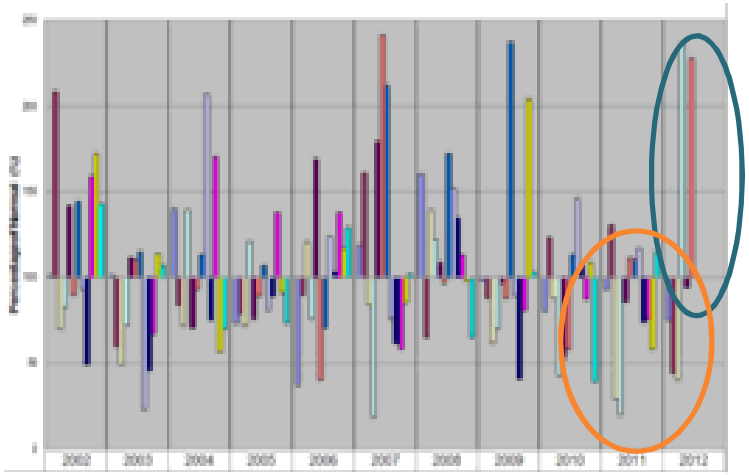


Rainfall Anomalies Wales (2002-2012)

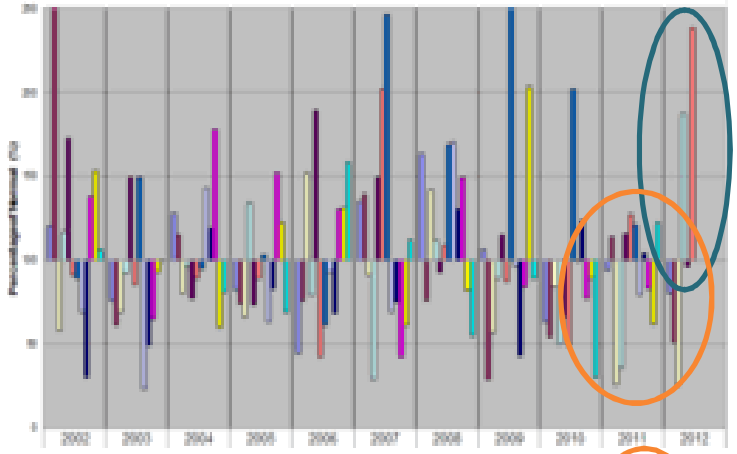


Rainfall Anomalies Scotland (2002-2012)

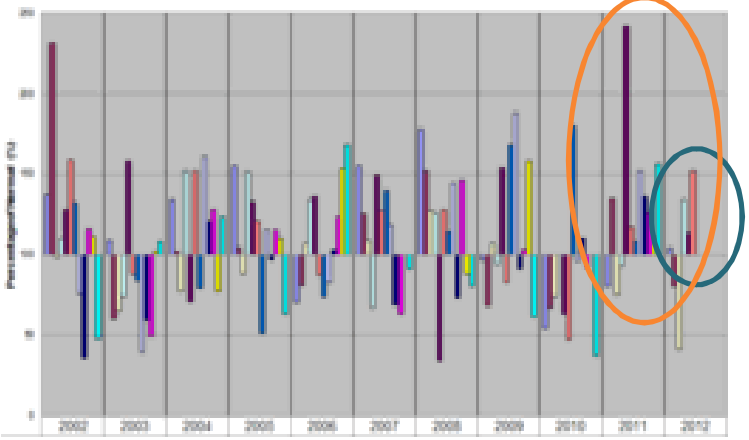




England



Wales



Scotland

Country, regional and local differences occur making general UK forecast predictions difficult

Network of synoptic and climate stations provide local daily data

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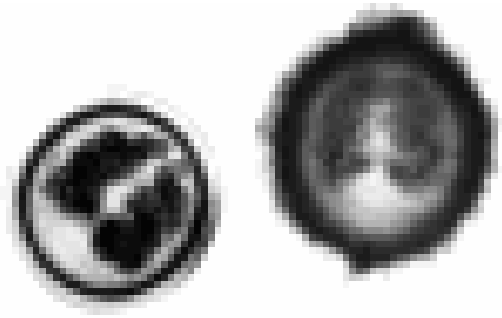
EFFECTS OF CLIMATE CHANGE

Milder Winters increase:

- Survivability of hibernating snails
- Shedding of cercariae in the following spring
- Increased infection in flocks in the spring

Wetter Summers increase:

- Levels of snail infection
- Numbers and early appearance of metacercariae on pasture



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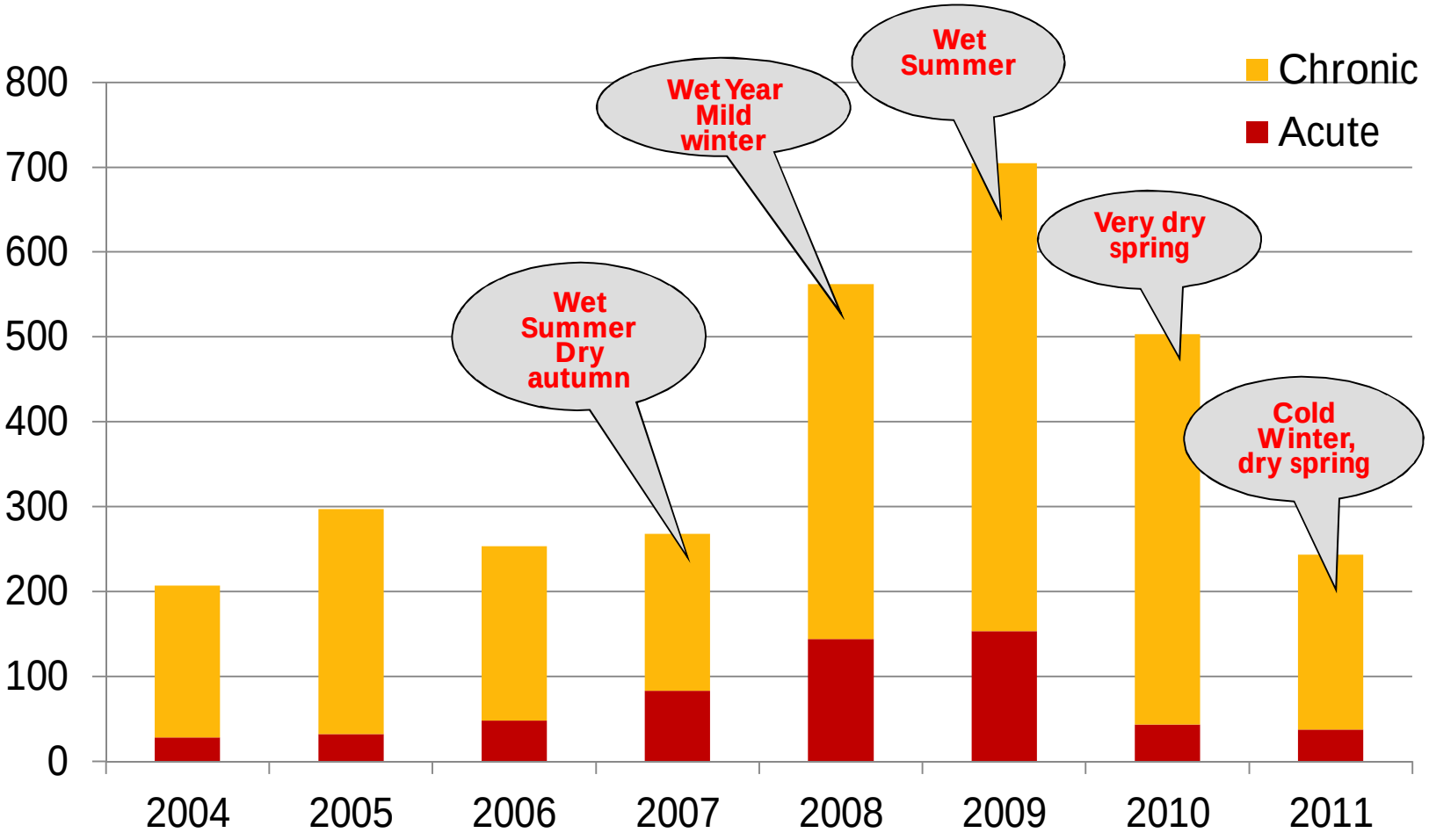
Reported Liver Fluke in Sheep



Veterinary Investigation Diagnosis Analysis (VIDA) figures of acute and chronic fasciolosis in sheep

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FLUKE FORECASTING

The "Ollerenshaw Method"

Developed at CVL using fluke prevalence and climate data from farms and meteorological stations

Seasonal index derived from rainfall, number of rain days and potential evapotranspiration and is interdependent with temperature

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The Ollerenshaw Index

- Fasciolosis risk value (Mt) calculated for each month and summated to give seasonal risk values
- Mt set to zero if mean monthly temperature <10ºC
- Initially set for May to October (<u>now</u> changing climate!?)
- Mt values capped at 100 as cap usually reached after 18 rain days
- Formula

Mt Fasciolosis risk value
n Number rain days/month
R Rainfall (mm/month)
P Potential evotranspiration

• Mt<300	little or no risk
• Mt=400	occasional losses
• Mt400-474	disease prevalent
• Mt>474	serious outbreaks

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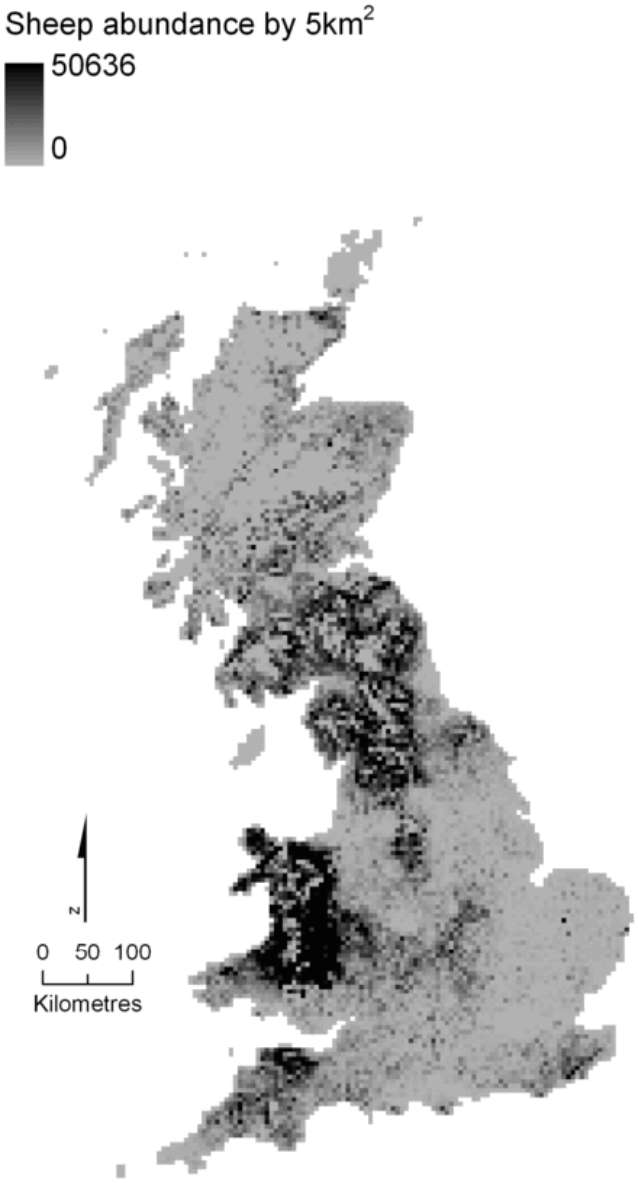
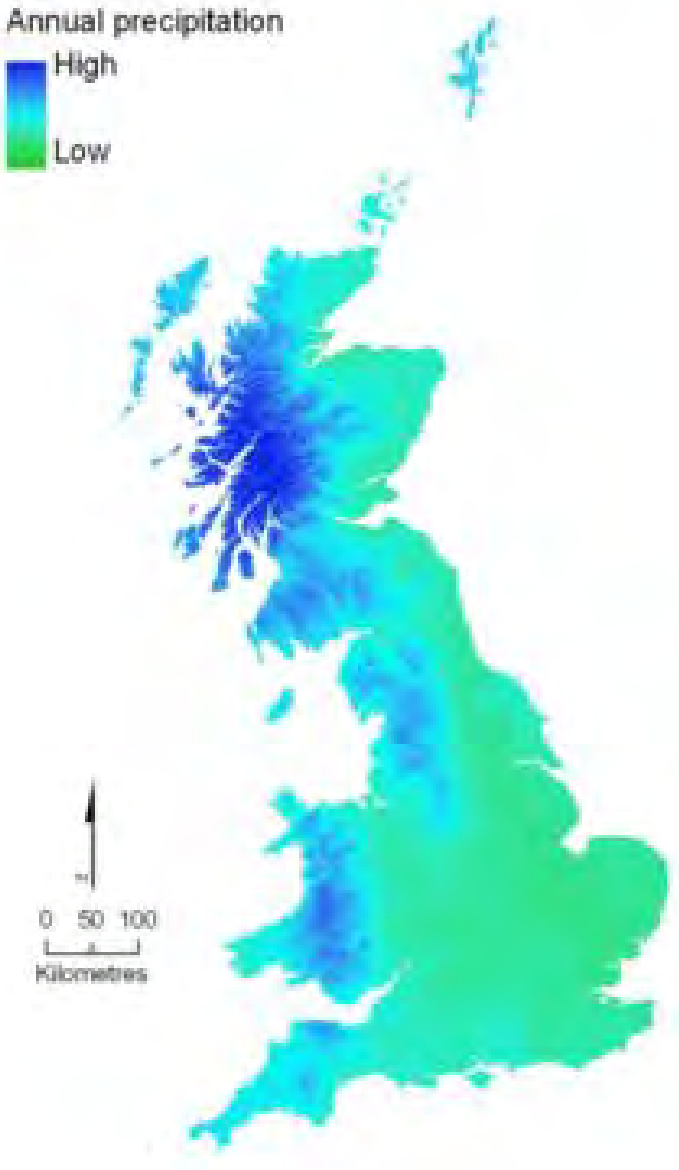
NADIS Fluke Forecasts

Predictive Modelling





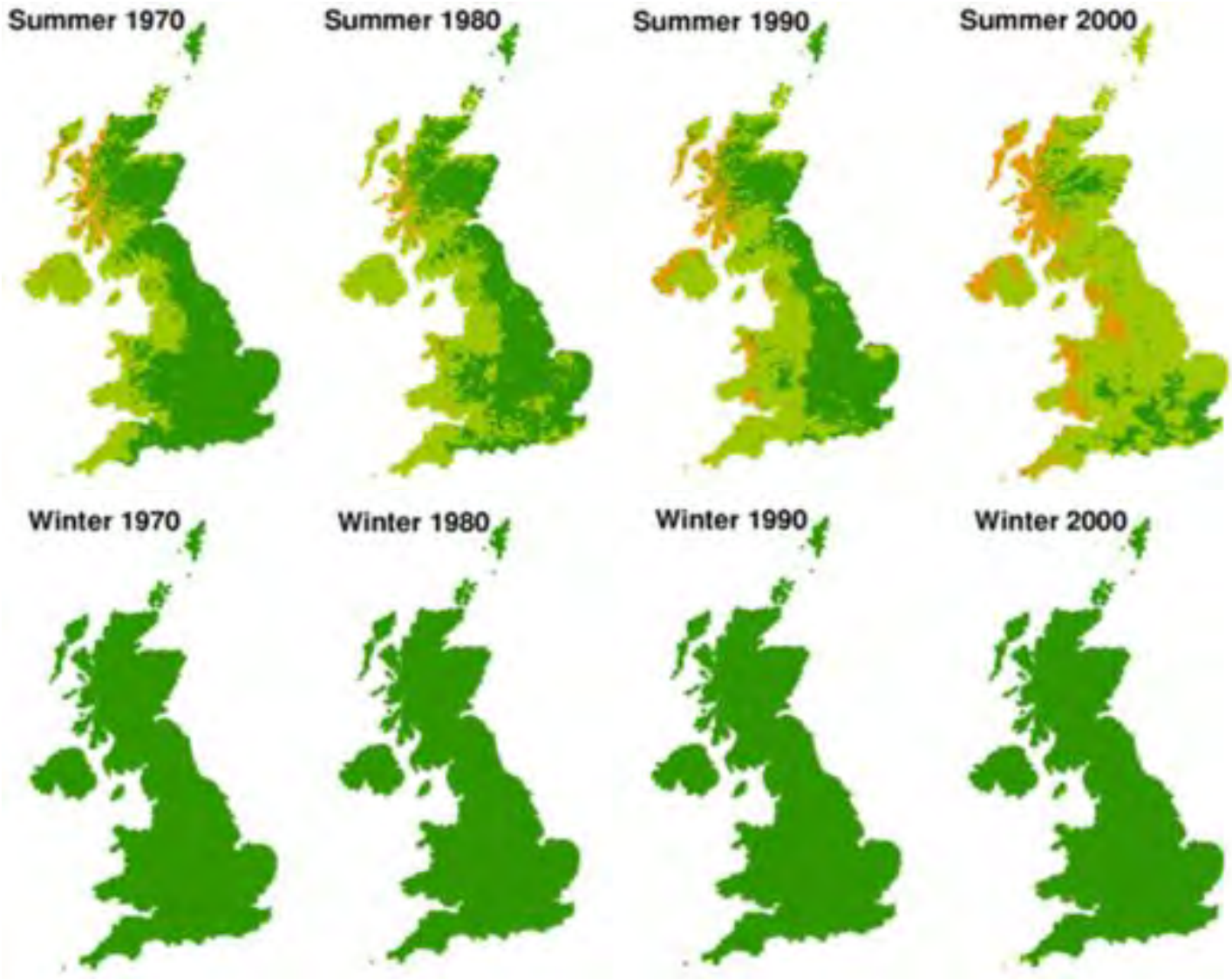
The regions of the UK used in comparing past and future risk

Code	Region
0	South West
1	South East
2	West Midlands
3	East Midlands
4	East of England
5	North West
6	Yorkshire and The Humber
7	North East
8	London
9	Northern Ireland
10	West Wales and The Valleys
11	East Wales
12	Highlands and Islands
13	South Western Scotland
14	Eastern Scotland
15	North Eastern Scotland

England = 0–8, Northern Ireland = 9, Wales = 10–11, Scotland = 12–15.
doi:10.1371/journal.pone.0016126.t001

Fox NJ, White PCL, McClean CJ, Marion G, et al. (2011) Predicting Impacts of Climate Change on *Fasciola hepatica* Risk. PLoS ONE 6(1): e16126. doi:10.1371/journal.pone.0016126
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0016126

Past change in fasciolosis risk



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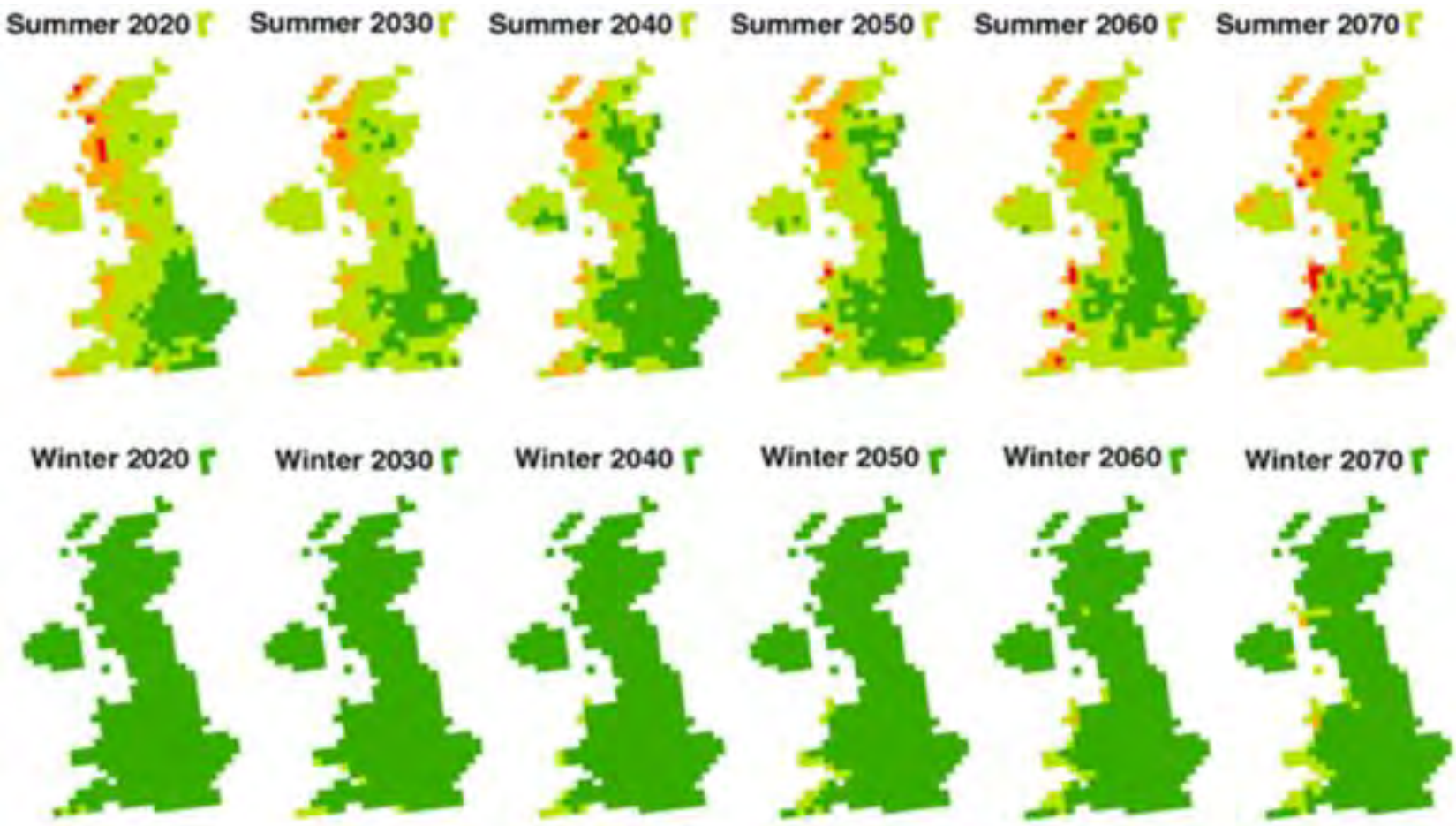
Projected change in fasciolosis risk



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http://www.plosone.org/article/info:doi/10.1371/journal.pone.0016126

What we need to do?

- Improved 'predictive' (not retrospective) surveillance
  - Snail monitoring?
  - Improved regional disease reporting via vet networks
- Regional/local weather monitoring
- Climatic and predictive modelling
- Model validation from disease reports
  - Laboratory diagnostics – methods?
  - On farm PM results
  - Abattoir findings – MHS?
- Needs to be centrally co-ordinated and reported

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LIVER FLUKE

Dairy Cows – Treatment Options

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FLUKICIDES

- Triclabendazole is active against adult fluke and immature fluke (>1 week)
- Closantel and Nitroxynil are active against adults and immature fluke (>6 weeks)
- Products containing oxyclozanide and albendazole (given at 1.5 times the worm dose) are only active against adult fluke

Chemical Group	Anthelmintic	Fluke Activity			Comments
		Adult	6-12 wks	1-6 wks	
Salicylanilides	Closantel	+	+	-	Pour-on and injection
	Oxyclozanide	+	-	-	Only in combination with levamisole in UK
Substituted phenols	Nitroxynil	+	+	-	
Benzimidazoles	Albendazole	+	-	-	
	Triclabendazole	+	+	+	
Sulphonamide	Clorsulon	+	-	-	Only in combination with ivermectin

WITHDRAWAL PERIODS

Anthelmintic	Withdrawal Periods	
	Meat	**Milk**
Closantel	28-49	Do not use in non-lactating dairy cows including pregnant heifers within 60 days of calving.
Oxyclozanide	5	Must not be used in cattle producing milk for human consumption.
Nitroxynil	60	Not for use in cattle producing milk for human consumption including pregnant animals intended for milk production.
Albendazole	14	Milk for human consumption must not be taken during treatment. Milk for human consumption may be taken from cows only after **60 hours** from the last treatment.
Triclabendazole	56	Do not administer to cattle producing milk intended for human consumption.
Clorsulon	66	Not for use in cattle producing milk for human consumption. Do not use in non-lactating dairy cows or pregnant heifers within 60 days of calving.

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CATTLE TREATMENTS

- Monitor herd regularly to determine the need for treatment:
  - *post-mortem* examinations when the opportunity arises,
  - from abattoir returns
  - with FECs
  - Milk or serum fluke ELISAs
- Acute fluke rare in cattle and majority of treatments are aimed at chronic fasciolosis (adult fluke)
- Important to use the appropriate flukicide for each situation
- Products containing albendazole have 60 hour milk withdrawal period.
- Dry period treatments and recommendations for use in pregnant non-lactating cattle vary
- “Fluke and worm” combination products should be discouraged as may lead to off-target selection for resistance

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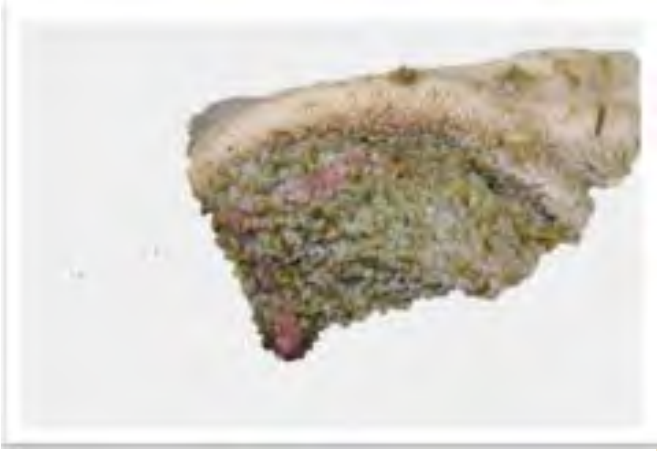
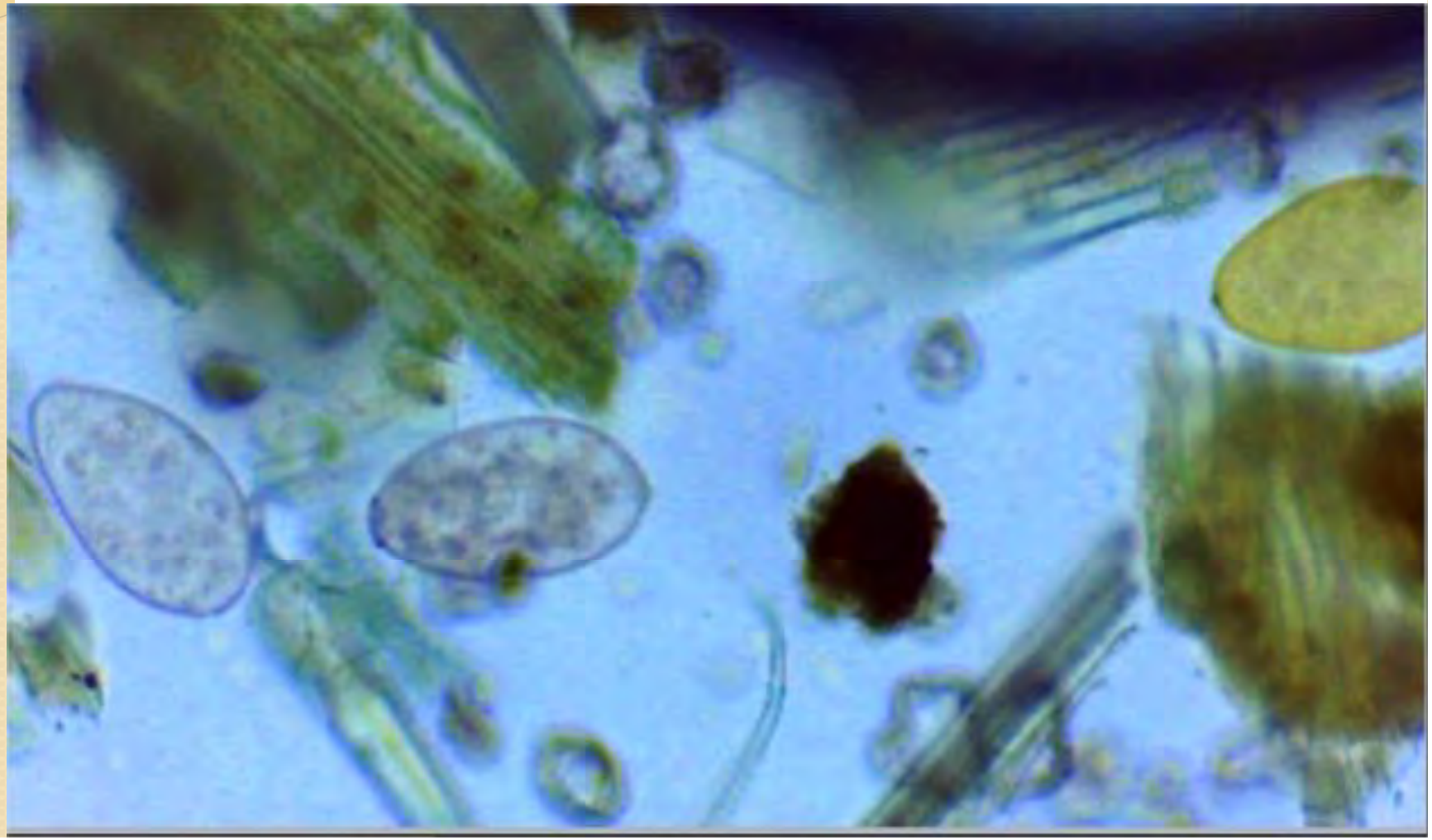
RUMEN FLUKE

- *Paramphistomum spp.* found in the rumen of cattle, sheep, deer and other ruminants
- Life cycle is similar to liver fluke and involves water snails
- Eggs are of similar size to *Fasciola*
- Adult fluke do not usually cause disease
- Immature flukes (in large numbers) may cause death
- Oxyclozanide only drug with reported activity



RUMEN FLUKE EGGS



The diagnosis of fasciolosis

Roger Daniel, AHVLA Carmarthen

AHVLA
Animal Health and Veterinary Laboratories Agency

The diagnosis of fasciolosis

- In the individual animal
- At herd/flock level
- Potential new tests
- TCBZ lack of efficacy
- AHVLA & SAC VIDA diagnostic data

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Post mortem examination

- Definitive diagnosis of acute and chronic fasciolosis
- Indicates degree of challenge
- Indicates previous infection
- Diagnosis of black disease

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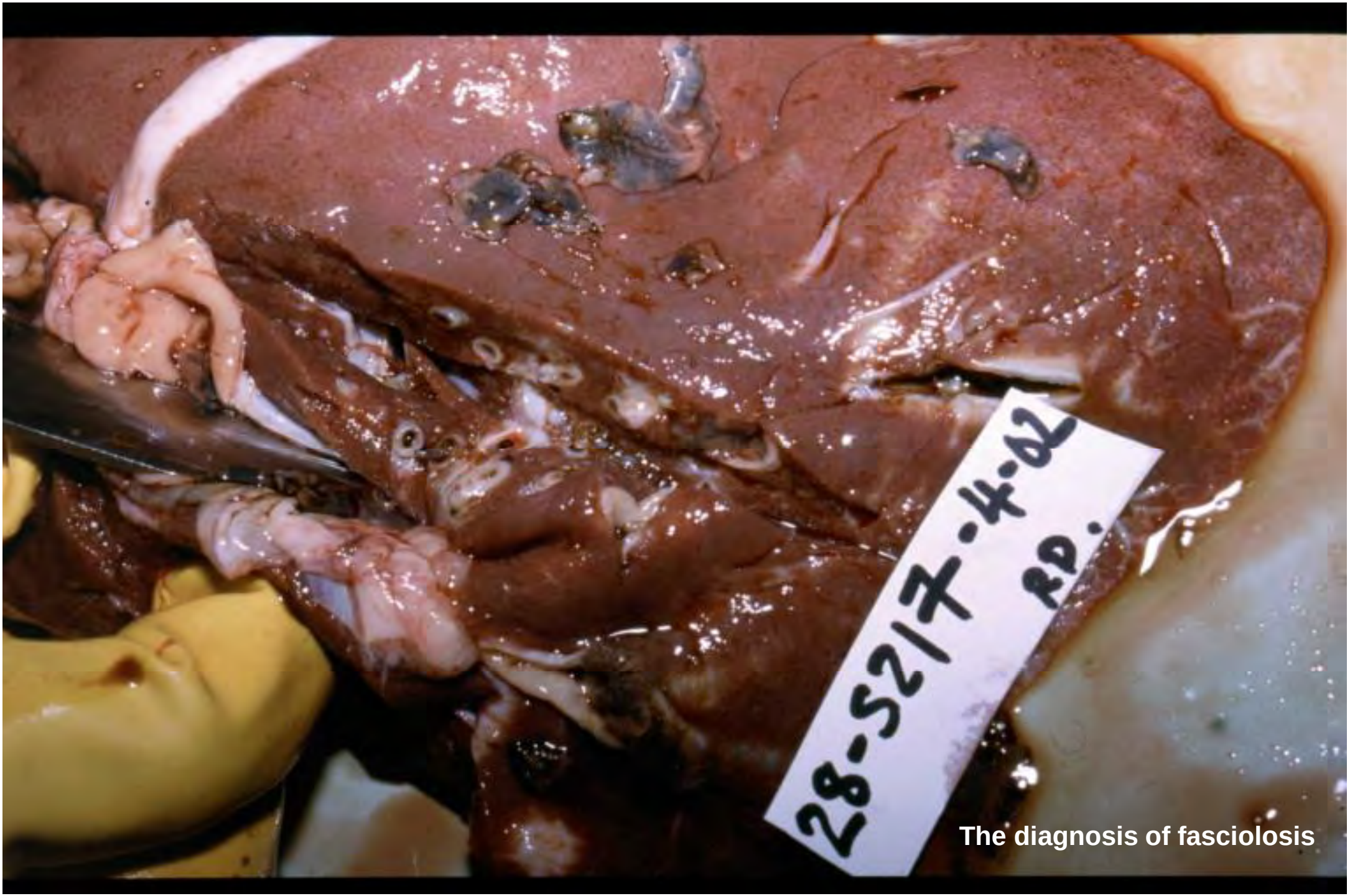

The diagnosis of fasciolosis

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Liver rejection data (fluke) 2010		Throughput	Liver condemnation	%	Estimated loss (£) from liver rejection only (sheep liver = £1.30, cattle liver = £4.00[1])
England	Cattle	1,547,151	306,499	19.81	£1,225,996
	Sheep	8,625,486	700,982	8.13	£ 911,277
Wales	Cattle	135,563	38,126	28.12	£152,504
	Sheep	3,672,596	165,877	4.52	£215,640
Scotland	Cattle	518,461	143,271	27.63	£573,084
	Sheep	1,463,044	128,560	8.79	£167,128

Figures from Phil Handley of EBLEX

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Faecal egg detection

- Sedimentation technique is method of choice
- Up to 40gm of faeces enhances diagnosis
- Sensitivity 30-60%, specificity 100%

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False negative results

- First 10-12 weeks of infection - no eggs are laid
- Low numbers shed in cattle faeces, 2-5 epg of faeces
- Undetectable in dilute scour samples

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False negative results - cattle

- **Intermittent shedding - eggs pool in gall bladder**
- **Over-dispersion of infection within herd**

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Clinical chemistry - Liver enzymes

- **Supportive tests only**
- **Raised liver enzymes (AST & GLDH) indicate hepatic cell damage**
- **Detectable from 13 days post infection**

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Clinical chemistry - Liver enzymes

- Raised γGT indicates bile duct damage
- Detectable from 40 days post infection

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Serology – Cattle only

- **Use fluke protease enzymes as antigen**
- **Little cross reaction with antigens of other internal parasites**

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Serology - Experimental findings

- Antibodies appear 2-4 weeks after infection
- Levels can persist for 6 months (Castro), or 11 months (Boulard) after removal of parasite
- Positive serology not an indicator of current infection

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Serology – Cattle only

- ***Levels can decline rapidly in some cases***
- **Indicates herd exposure**

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Coproantigen ELISA in faeces

- Detects fluke protein in faeces
- Can detect infection in the prepatent period, before egg laying commences
- Not employed by AHVLA, reliability in cattle faeces?

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Id.	Fluke eggs per gram in 40g sedimentation	Room temp Day 1	Fridge Day 1	Room temp Day 2	Fridge Day 2	Room temp Day 3	Fridge Day 3	Room temp Day 4	Fridge Day 4
A	0.225	0.206	0.332	0.319	0.369	0.244	0.357	0.197	0.209
B	0.1	0.003	0.010	0.011	0.017	0.009	0.006	-0.010	-0.064
C	0.6	0.091	0.069	0.107	0.105	0.082	0.064	0.055	0.070
D	3.8	0.206	0.214	0.245	0.301	0.189	0.304	0.145	0.204
E	5.5	0.079	0.076	0.113	0.108	0.067	0.102	0.023	0.096
F	0.05	0.009	0.028	0.022	0.053	0.005	0.056	0.002	0.012
G	1.475	0.108	0.176	0.084	0.267	0.054	0.259	0.048	0.191
H	0.05	0.141	0.202	0.121	0.182	0.080	0.139	0.022	0.088
I	0.475	0.068	0.083	0.089	0.098	0.067	0.079	0.021	0.069
J	3.05	0.212	0.268	0.233	0.213	0.221	0.182	0.180	0.145

False Negatives

True positives

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Coproantigen ELISA validation

- Would require comparison between liver pathology, egg count and coproantigen result
- Does the coproantigen protein level decline during postage to the laboratory

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What tests & when

- PM - any time of year
- PM – can inform treatment
- FE detection requires patent infection, autumn and early winter for untreated animals
- During housing to check efficacy of flukicide treatment

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Herd/Flock level diagnosis of fasciolosis

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AHVLA Bulk Tank ELISA for dairy herds

- Indicates herds where > 25% of cows are positive for fluke antibody
- 25 % is economic level of infection?
- Used in epidemiological studies and risk assessments of infection for dairy herds

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Two bulk tank milk surveys in dairy herds, 2002-3 & 2006-7

- **2003**, samples obtained from 623 herds in England and 445 in Wales
- Number of herds infected - 47% in England, and 86% in Wales

- **2006-7**, samples obtained from 3,130 herds
- Number of herds infected – 76% in England, 84% in Wales

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Composite faeces samples – cattle and sheep

- Faeces taken from 10 dung pats
- Submit separately
- 5 gm taken from each of 10 samples at laboratory
- Results Pos = fluke eggs seen
  Neg = No fluke eggs seen

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Herdsure protocol – use of composite faeces samples

- Samples collected for 4 composite fluke egg detection tests from at-risk groups on farm
- By testing 4 pools of 10 samples, there is a 95% confidence level of detecting one positive animal if the within herd prevalence is 20%

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Use of PCR technology

- Detect fluke DNA in faeces of infected animals
- Detect fluke DNA in infected snails
- Detect fluke DNA (metacercariae) on herbage

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A composite faecal egg count reduction test to detect resistance to triclabendazole in *Fasciola hepatica*

R. Daniel, J. van Dijk, T. Jenkins, A. Akca, R. Mearns, D.J.L. Williams

Veterinary Record

online

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Composite fluke egg count reduction test

- Failure of TCBZ treatment identified on 7 of 25 sheep farms
- Now validated using two composite samples only per farm
- Robust method, not requiring individual sheep faeces samples

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AHVLA & SAC VIDA diagnostic data

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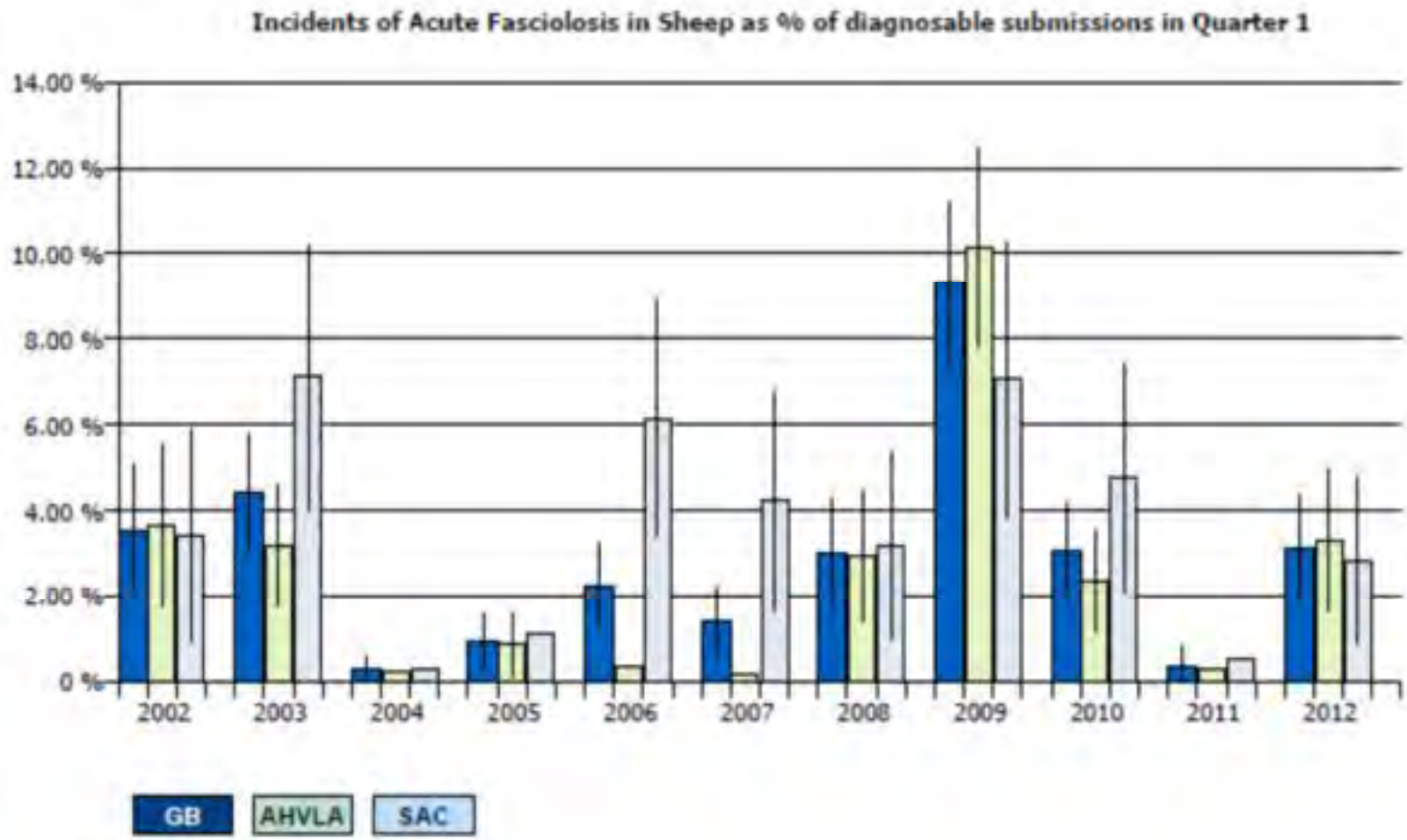
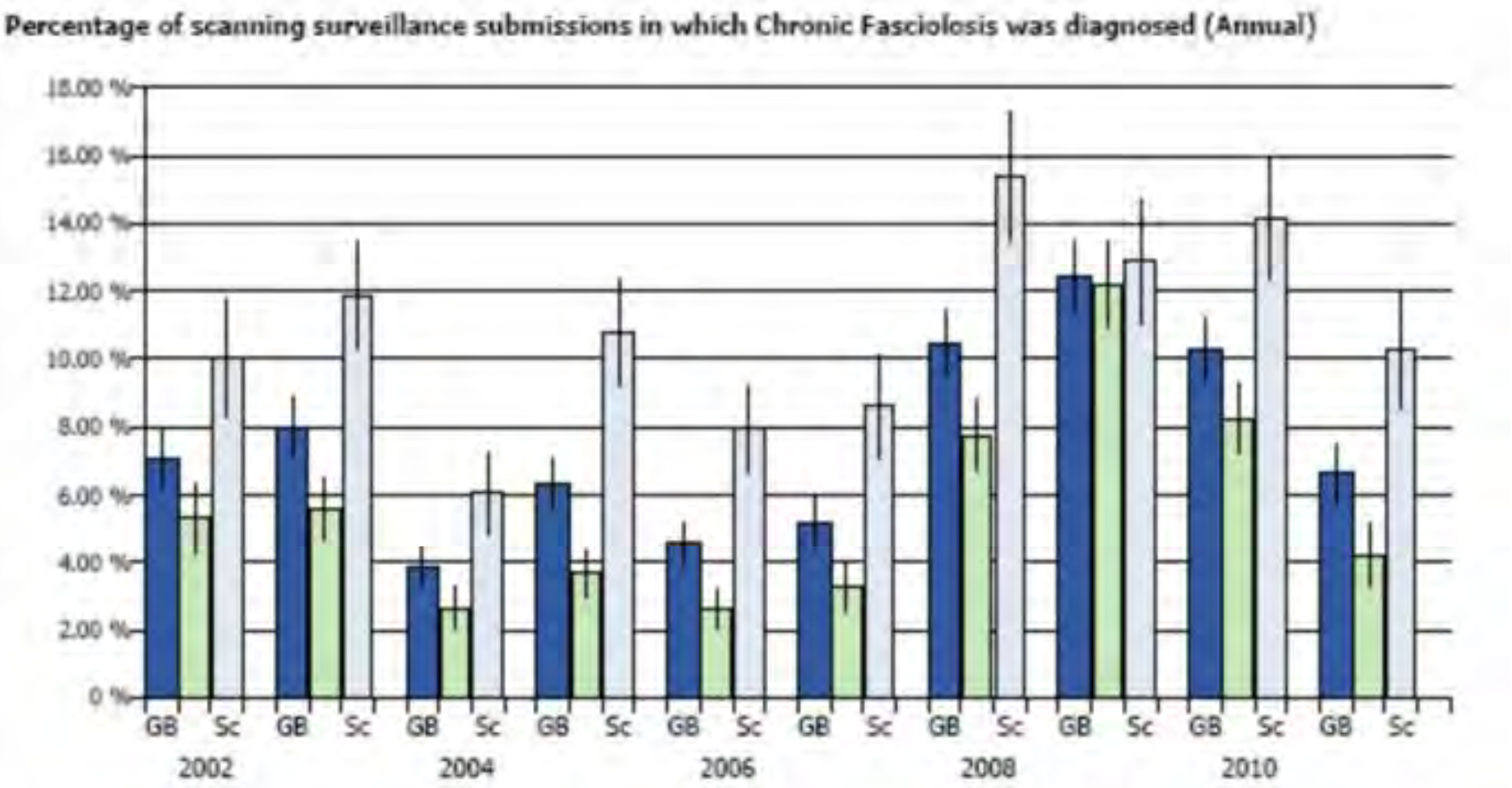
VIDA Data for acute fasciolosis in sheep for England/Wales & Scotland Quarter 1 2002-2012



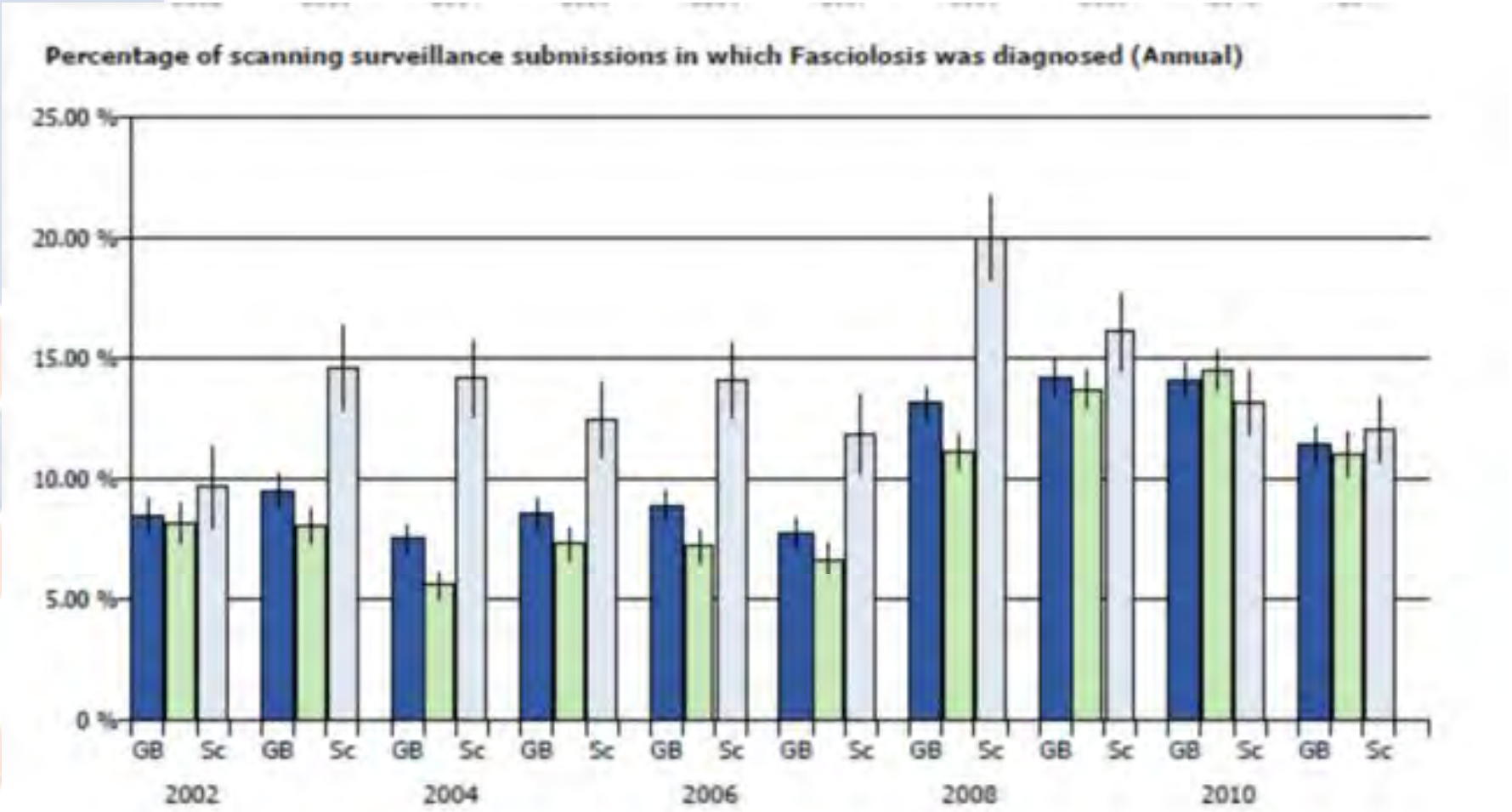
Agency

VIDA Data for chronic fasciolosis in sheep for England/Wales & Scotland from 2002-2011



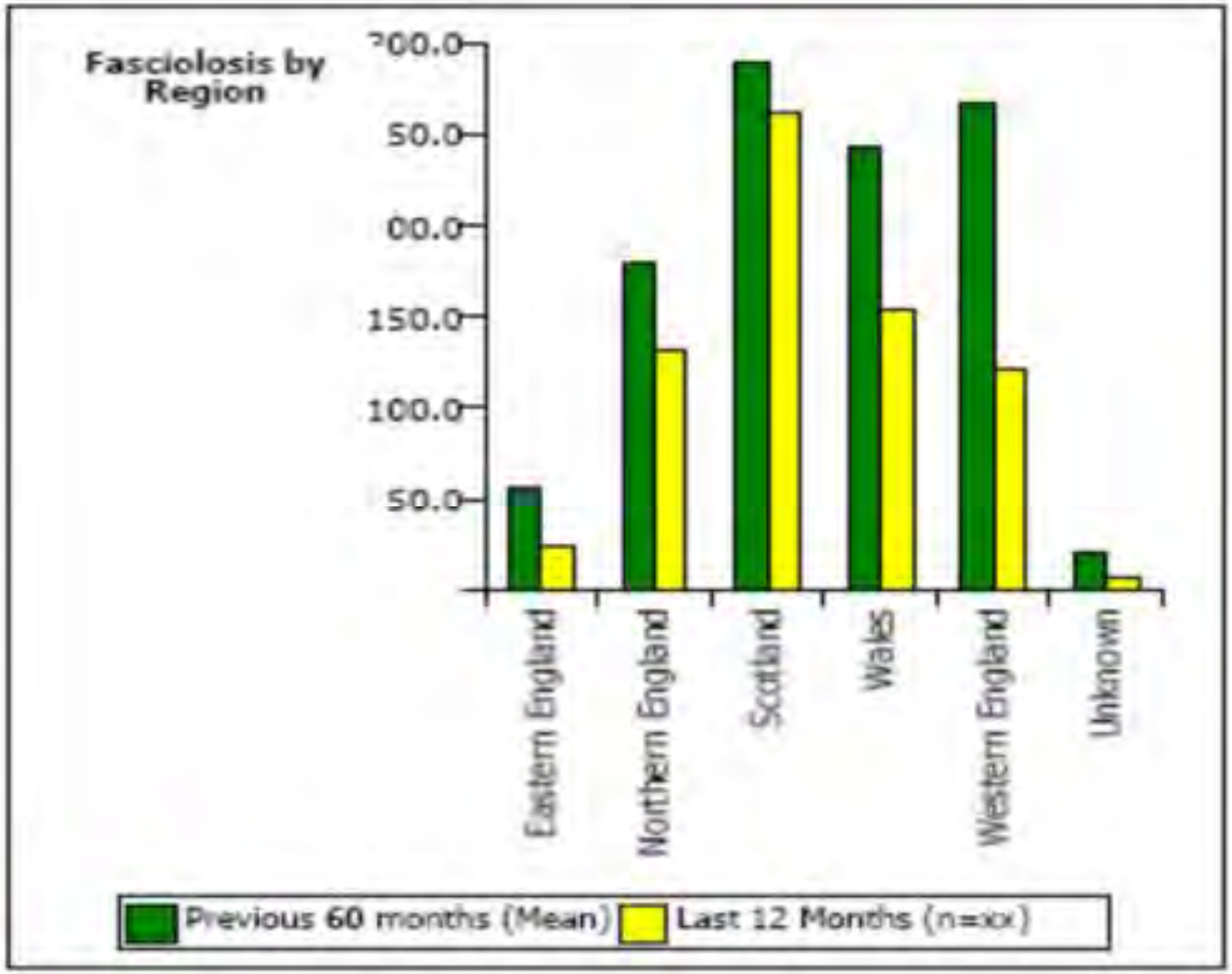
Animal Health and Veterinary Laboratories Agency

VIDA Data for fasciolosis in cattle for England/Wales & Scotland 2002-2011



Animal Health and Veterinary Laboratories Agency

Number of diagnoses of fasciolosis by region for last 12 months compared to average of previous 60 months



AHVLA
Animal Health and Veterinary Laboratories Agency

Abattoir rejection data

- **Does not distinguish between fasciolosis and *Taenia hydatigena* infection**
- **In dual infections, only one maybe recorded**
- **FSA do not record farm of origin of stock when livers rejected**
- **Does farmer get paid if liver not rejected?**

AHVLA
Animal Health and Veterinary Laboratories Agency

Survey of anthelmintic resistance in liver fluke and gastrointestinal nematodes in sheep in Northern Ireland.

Bob Hanna[1], Connor McMahon[2], Sharon Ellison[2], Hillary Edgar , Jason Barley[1], Desmond Irwin[3], Gerry Brennan[2], Ian Fairweather[2].

[1]DSIB, VSD, AFBI, Stormont, Dundonald, Belfast BT43SD
[2]Queen's University, School of Biological Sciences, Belfast BT9 7BL
[3]Agricultural Research Institute, AFBI, Hillsborough BT266DR, Co. Down.

Working in partnership with industry

- Anthelmintic resistance in gastrointestinal nematode and liver fluke populations is well-known throughout the world

- Numerous reports and surveys internationally, including European countries, UK and Ireland

- Current situation in Northern Ireland is less well-known, despite the fact that ruminant production represents a very significant proportion of our GDP

- To rectify this, the Parasitology Sections of VSD, AFBI and the School of Biological Sciences at Queen's University undertook several joint surveys in 2011

- Province-wide questionnaire relating to anthelmintic use and farm management on sheep and cattle farms : 300 returns

- On-farm investigation of efficacy of anthelmintic in use on 93 sheep farms drawn from the 300

- Comparative field trial on various flukicides and nematocides on 13 well-managed sheep farms routinely participating in AFBI-led productivity research projects (HILLSBOROUGH PROJECT)

Liver Fluke: Anthelmintic Resistance Survey 2011

Working in partnership with industry

Hillsborough anthelmintic resistance project.

- Thirteen farms were visited during the winter and early spring
- On each farm individual rectal faeces samples were collected from approximately 60 sheep, i.e. 3 groups of 20 animals each (PRE-DOSE)
- The sheep were individually marked and then dosed to weight with triclabendazole (fasinex), closantel (flukiver) or nitroxynil (trodax)
- Care was taken to ensure accuracy of dosing and efficacy of the drench
- Three weeks later POST-DOSE faeces samples collected from as many as possible of the sheep sampled previously
- Faecal Egg Counts were carried out on all the faeces samples

Results of FECs: Fluke burdens

- 1 farm was **free of fluke**, and on 2 other farms only 1 or 2 eggs were found in several animals, suggesting freedom from current infection

- 4 farms had **low** fluke burdens <10/60 animals infected, counts <20 EPG

- 2 farms had **moderate** fluke burdens 30/60 animals infected, counts <100 EPG

- 4 farms had **heavy** fluke burdens 55+/60 animals infected, counts up to and > 700EPG

Results of FECs: Failure of TCBZ efficacy

- On the 4 farms with **heavy** fluke burdens TCBZ (fasinex) treatment was **ineffective** in reducing FECs, but closantel (flukiver) and nitroxynil (trodax) reduced the FECs to zero in the 3-week post-infection period

- On one farm with a **moderate** fluke burden, TCBZ appeared **ineffective** in reducing FECs, but closantel and nitroxynil were effective

- On one farm with a **moderate** fluke burden, TCBZ appeared to be **partly effective** but the FECs were too low to allow meaningful interpretation

- On the farms with **low** fluke burdens, the **FECs were too low** to give statistical validity to the results, but TCBZ may remain at least partially effective and in these cases

Results:

Example of a farm with a high fluke burden

TCBZ ineffective

Panacur			Flukiver			Tredax		
Sample LD	Pre	Post	Sample LD	Pre	Post	Sample LD	Pre	Post
1	36	27	1	45	0	1	343	0
2	17	20	2	155	0	2	>200	0
3	309	323	3	31	0	3	2	0
4	28	800	4	20	0	4	0	0
5	11	4	5	41	0	5	48	0
6	51	104	6	205	0	6	347	0
7	>500	513	7	5	0	7	43	0
8	50	105	8	180	0	8	>200	0
9	83	82	9	200	1	9	>200	0
10	>500	486	10	450	0	10	11	0
11	>500	405	11	300	0	11	2	0
12	>500	211	12	85	0	12	0	0
13	52	58	13	41	0	13	13	0
14	>800	347	14	204	0	14	14	0
15	>200	503	15	113	0	15	0	0
16	34	4	16	97	0	16	179	0
17	1	0	17	6	0	17	344	0
18	3	0	18	0	0	18	133	0
19	0	0				19	>300	0

N/S = No Sample

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Results:

Example of a farm with a moderate fluke burden

TCBZ ineffective

Fasinex			Flukiver			Trodax		
Sample I.D	Pre	Post	Sample I.D	Pre	Post	Sample I.D	Pre	Post
1	0	0	1	0	0	1	2	0
2	11	11	2	1	0	2	0	0
3	0	1	3	0	0	3	10	[illegible]
4	1	6	4	0	0	4	31	0
5	0	1	5	0	0	5	0	0
6	0	0	6	1	[illegible]	6	69	0
7	0	1	7	0	0	7	1	0
8	25	28	8	0	0	8	10	[illegible]
9	0	0	9	4	0	9	2	0
10	134	[illegible]	10	1	0	10	0	[illegible]
11	0	0	11	0	0	11	30	0
12	0	0	12	41	0	12	4	0
13	0	0	13	0	0	13	0	0
14	0	[illegible]	14	25	0	14	3	0
15	1	0	15	0	0	15	0	0
16	0	4	16	0	0	16	0	0
17	2	4	17	27	0	17	2	[illegible]
18	3	0	18	0	0	18	13	0
19	0	0	19	15	0	19	72	0
20	0	0	20	0	[illegible]	20	37	0

N/S = No Sample

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Results:

Example of a farm with a moderate fluke burden

TCBZ partially effective ?

Fasinex			Flukiver			Tredax		
Sample I.D	Pre	Post	Sample I.D	Pre	Post	Sample I.D	Pre	Post
1	0	0	1	0		1	0	0
2	0	[illegible]	2	1	0	2	1	0
3	11	[illegible]	3	0	0	3	0	0
4	0	0	4	0	0	4	0	[illegible]
5	0	0	5	1	0	5	0	0
6	1	[illegible]	6	1	0	6	4	4
7	1	0	7	1	0	7	2	[illegible]
8	0	0	8	1	0	8	5	[illegible]
9	0	0	9	0	0	9	19	2
10	2	0	10	1	0	10	0	0
11	0	0	11	0	0	11	0	0
12	0	0	12	0	0	12	0	0
13	9	0	13	0	0	13	75	0
14	0	0	14	1	0	14	95	0
15	0	0	15	5	[illegible]	15	13	0
16	2	1	16	4	0	16	2	0
17	0	0	17	0	[illegible]	17	3	[illegible]
18	0	0	18	0	0	18	1	[illegible]
19	0	0				19	0	0
20	0	0						
21	1	0						

N/S = No Sample

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Supplementary tests:

Concerns regarding the use of FECRT for diagnosis of anthelmintic resistance in fasciolosis

- Eggs may still remain trapped in the gall bladder after flukes are removed (false positives)
- Pre-patent flukes will be missed (false negatives)
- Temporarily block of egg production without completely removing the flukes (false negatives)
- Under-dosage, faulty equipment, reduced metabolism, inadequate diagnostic method (sedimentation versus floatation)
- Supplementary tests are desirable to confirm true resistance
- Coproantigens
- Histology

Coproantigens

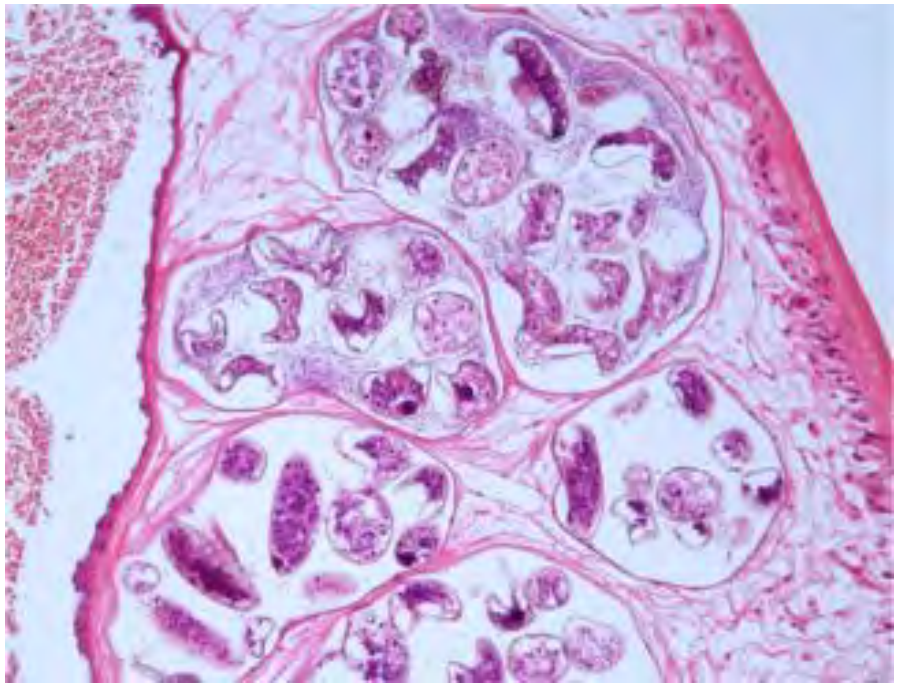
- Digestive enzymes from fluke gut
- Used to digest blood meal
- Eliminated into bile ducts and present in host faeces
- Detected by an ELISA method
- Supported the findings from FECRT on all five farms with heavy or moderate fluke burdens where TBZ treatment failed
- Post-treatment faeces samples from nitroxynil and closantel-treated sheep were negative, while post-treatment samples from TCBZ-treated sheep remained positive
- Where pre-treatment FECs were very low, coproantigens tended to be negative, perhaps indicating that no active flukes were present

Histology test for TCBZ resistance

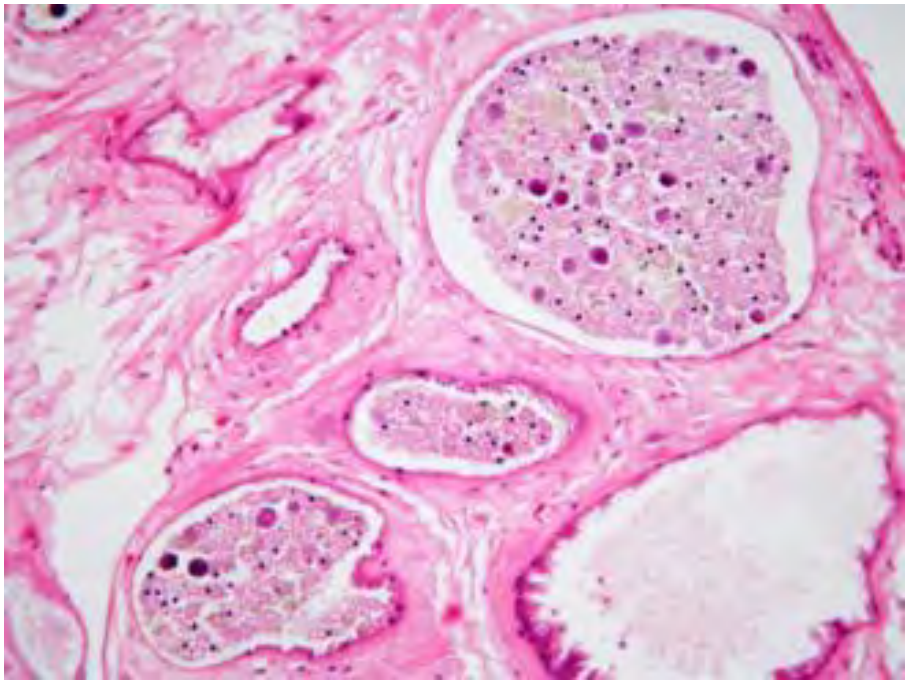
- In 3-day treated resistant flukes the eggs in the uterus are well-shelled, and spermatozoa are also present
- In the uterus of 3-day treated sensitive flukes no shelled eggs and no spermatozoa are present, only loose vitelline cells, shell debris and free oocytes

TCBZ 3 days

Resistant



Sensitive

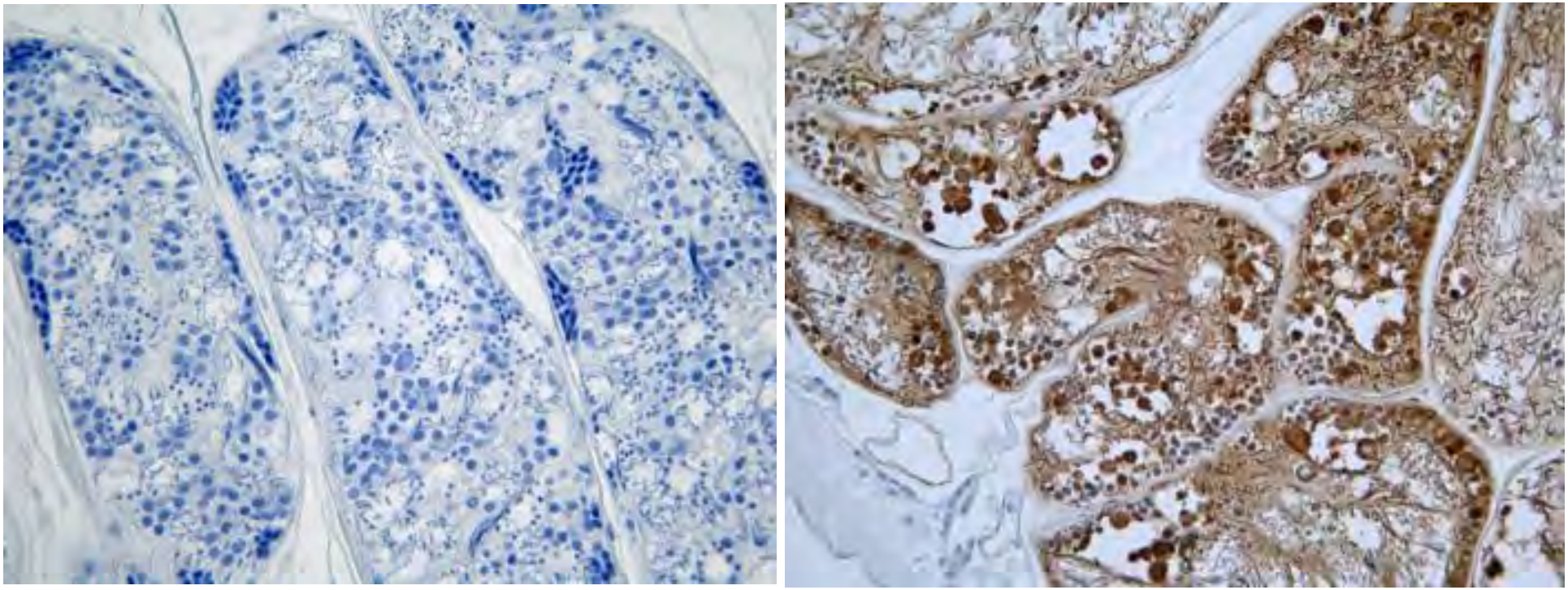


In situ hybridisation test

- Testis tubules of treated TCBZ-sensitive flukes are positive for endonuclease activity, demonstrating apoptosis and cytolytic activity.

3 of the 5 farms with FECRT and CA evidence of TCBZ-resistance were tested by histological methods, and the flukes were found to be resistant

Resistant **TCBZ 3 days *in vivo*** **Sensitive**



What to do ?

- If FECs indicate that **no fluke** are present, do not dose
- If fluke burden is **low** use closantel in spring to help prevent pasture contamination
- If fluke burden is **moderate** and there is **TCBZ resistance**, dose with closantel in spring and again in November and in the New Year
- If fluke burden is **moderate** and **TCBZ is partially effective**, dose with closantel in spring and with TCBZ in autumn to help prevent acute fluke damage
- If fluke burden is **high** and there is **TCBZ resistance**, dose with closantel in spring, and again in late September, mid-November and the New Year
- Grazing management is important. For example, graze wet areas where snails are likely to be present in early summer (before metacercariae are shed)

Use of flukicides against fasciolosis in sheep in Northern Ireland

Name of anthelmintic	2005	2011
	%	%
Triclabendazole	78.10 (0.21)	34.12 (1.16)
Closantel	9.48 (2.62)	53.76 (20.84)
Nitroxynil	7.81 (0.0)	10.15 (0.00)
Oxyclozanide	0 (0.0)	1.98 (0.00)

***ie move away from triclabendazole**

Diagnosis, diagnosis, diagnosis!

- Three categories:
- 1. Diagnosis of infection *per se*.
- 2. Diagnosis of drug efficacy.
- 3. Diagnosis of drug resistance.

Diagnosis of infection

- 1. Detection of eggs in faeces.
- 2. Liver enzymes.
- 3. Haematological parameters.
- 4. Serum antibodies via ELISA's.
- 5. Bulk-tank milk ELISA's.
- 6. Coproantigen ELISA.
- 7. Radiographic techniques (ultrasonography, magnetic resonance imaging, computer tomography).

Diagnosis of drug efficacy

- 1. Faecal Egg Count Reduction Test (FECRT).
- 2. Fluke counts (via controlled test/trial).
- 3. Coproantigen Reduction Test (CRT).
- 4. ??

Diagnosis of drug (TCBZ) resistance

- 1. FECRT.
- 2. Fluke counts (via controlled test/trial).
- 3. ??

Development of new tests:

- Coproantigen Reduction Test (CRT).
- Egg Hatch Assay (EHA).
- Histology.

2. Development of *in vitro* egg hatch assay

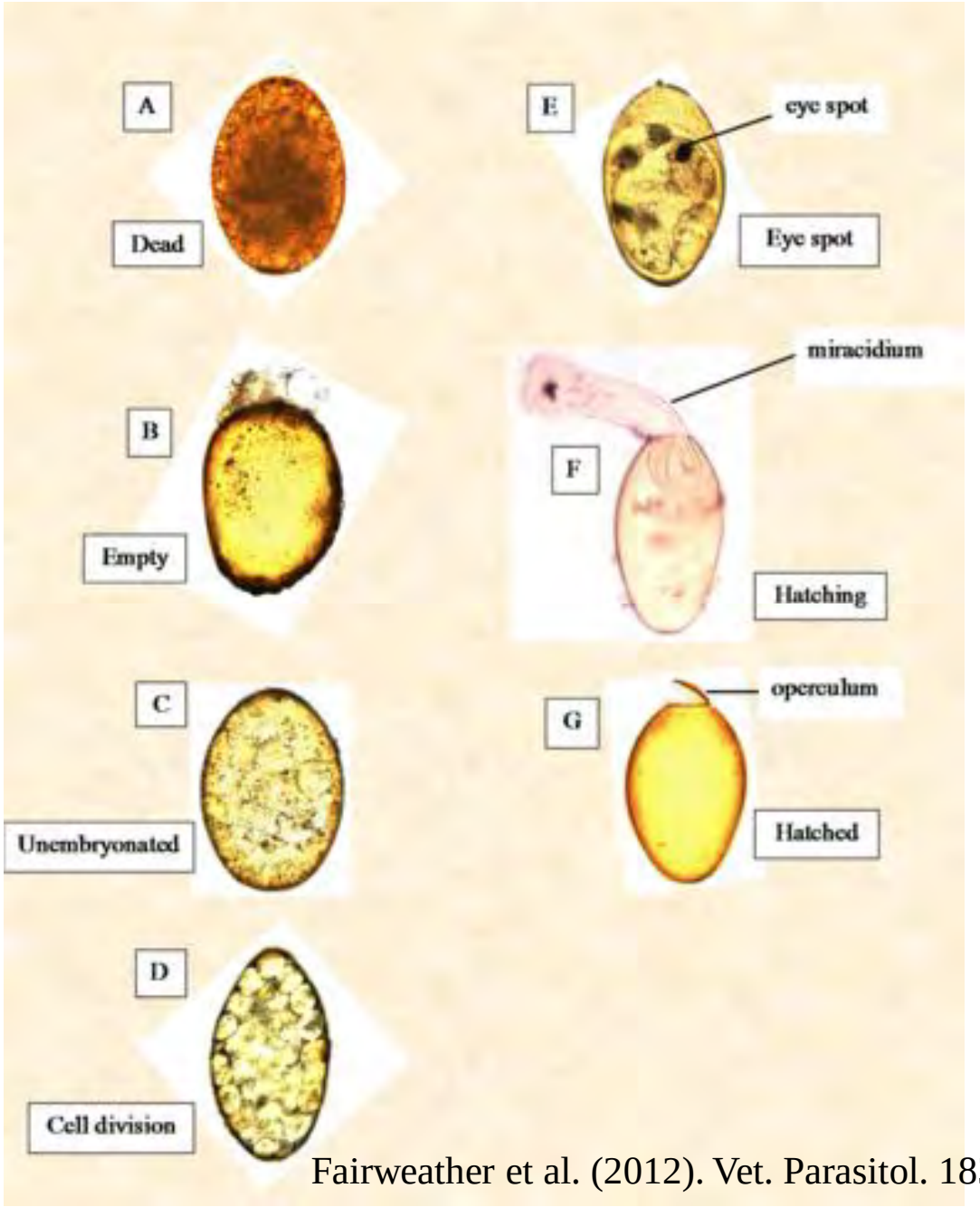
AIMS

- To determine the concentration of TCBZ.SO that prevents the hatching of 99% of eggs from a TCBZ-susceptible isolate.
- This is the so-called "discriminating dose".
- To compare the response of eggs from TCBZ-susceptible and TCBZ-resistant flukes.
- To evaluate the response of eggs from unknown field isolates of fluke.

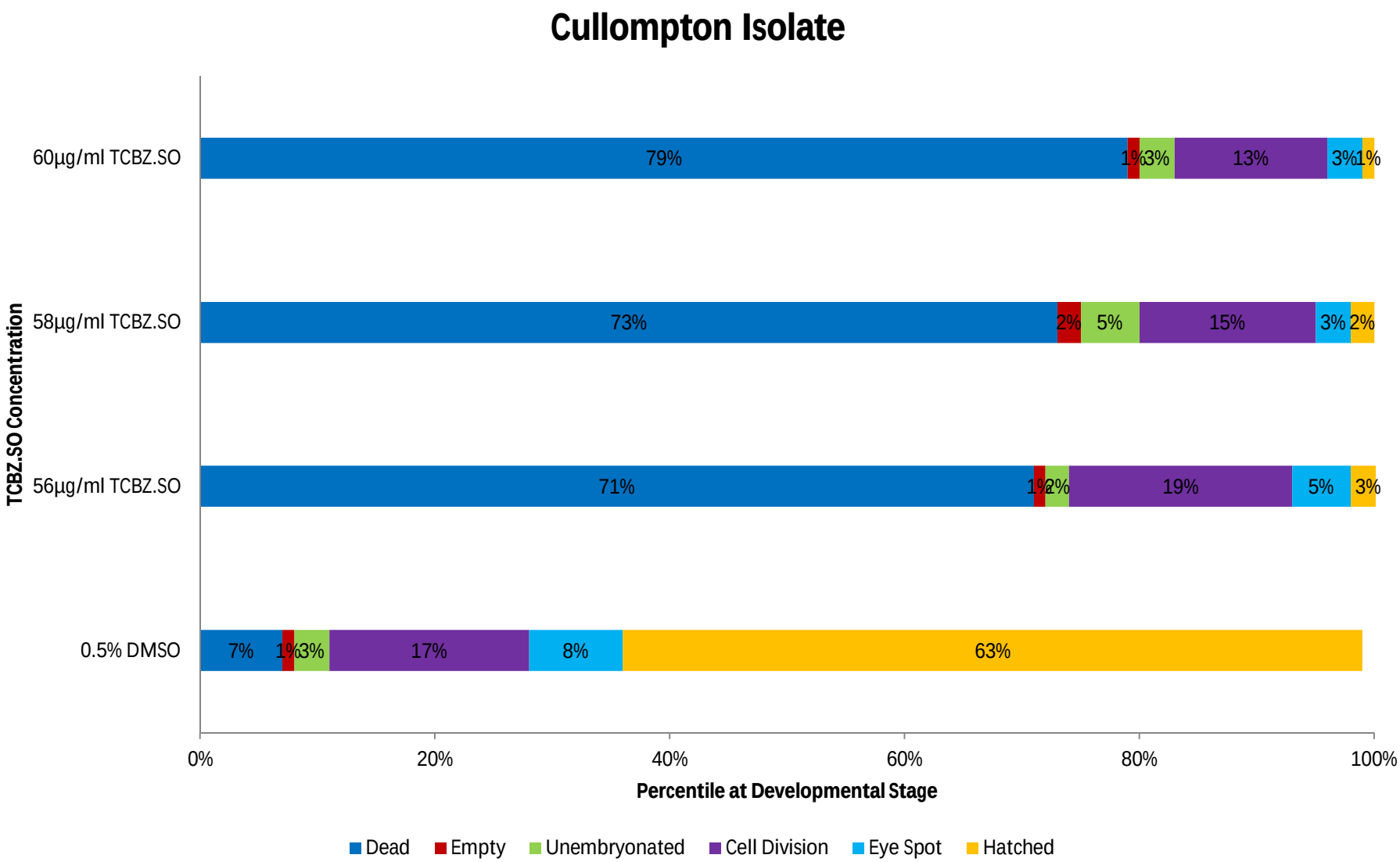
Egg Hatch Assay

- Discriminating dose set with the reference TCBZ-susceptible Cullompton isolate.
- Tested against the TCBZ-resistant Dutch and Patagonia isolates: assay confirmed their status.
- Tested against the Leon isolate, originally reported to be TCBZ-resistant.
- Assay showed that isolate was TCBZ-susceptible, confirming our efficacy data.

Different stages in egg development



Fairweather et al. (2012). Vet. Parasitol. 183, 249-259.



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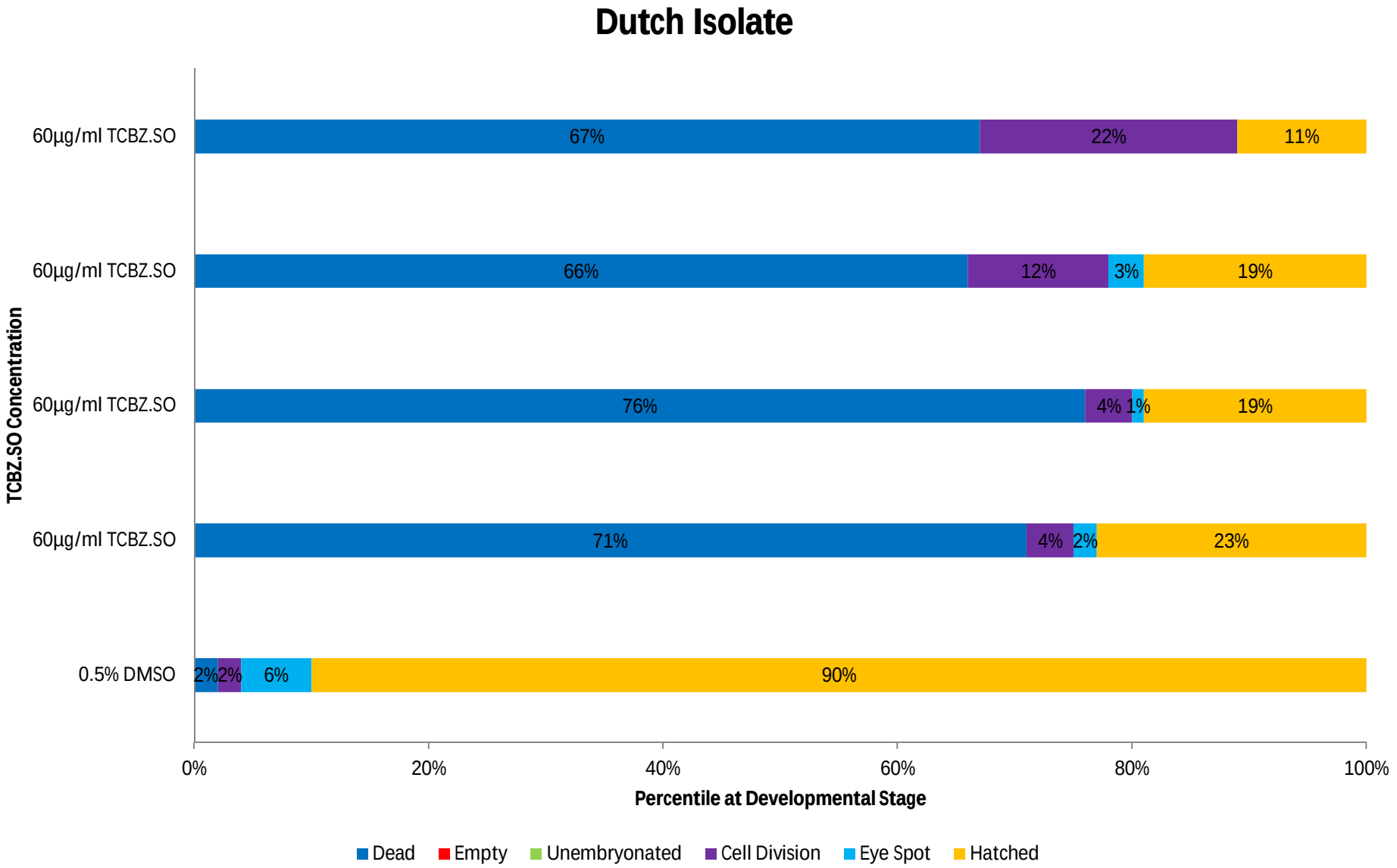

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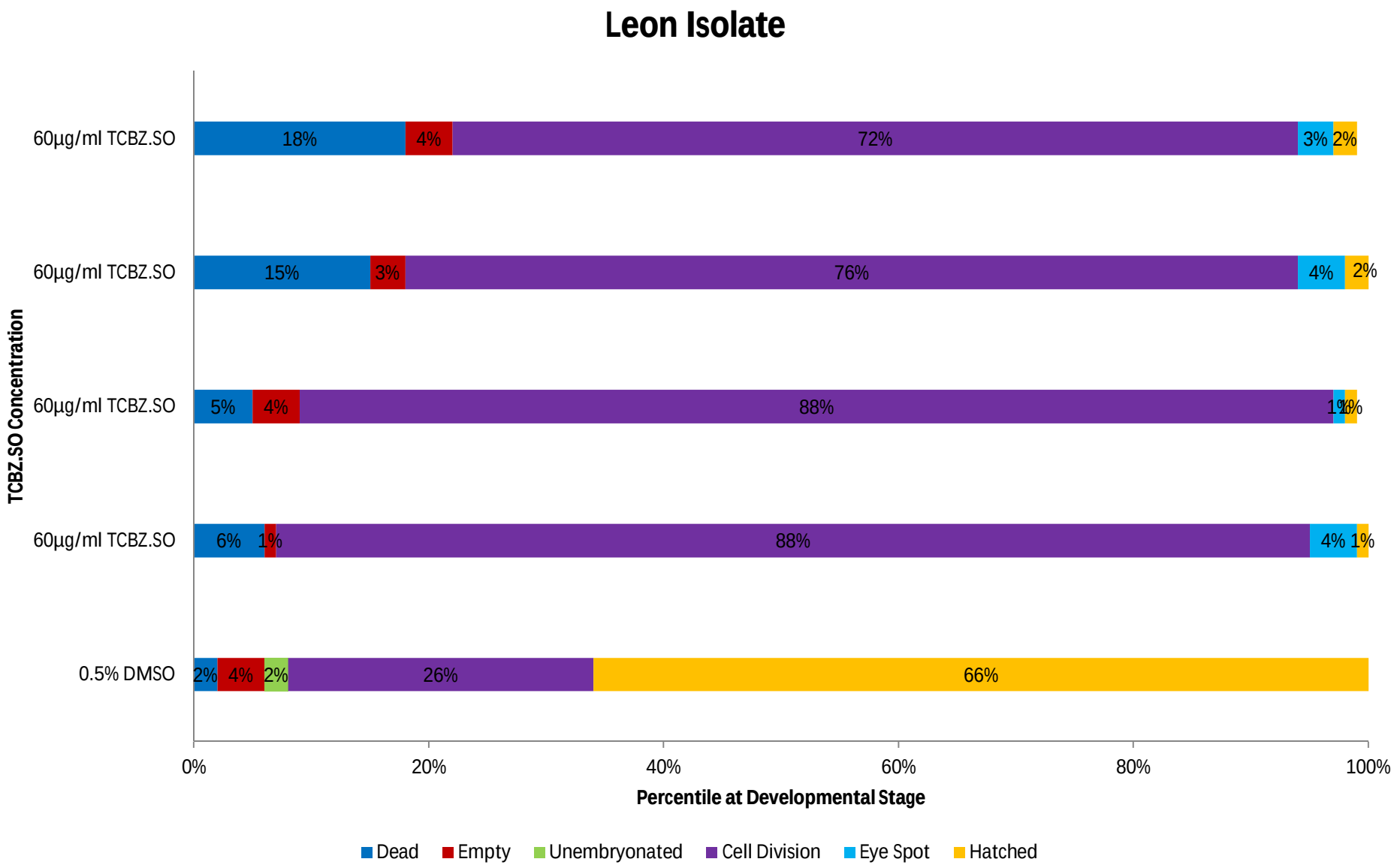
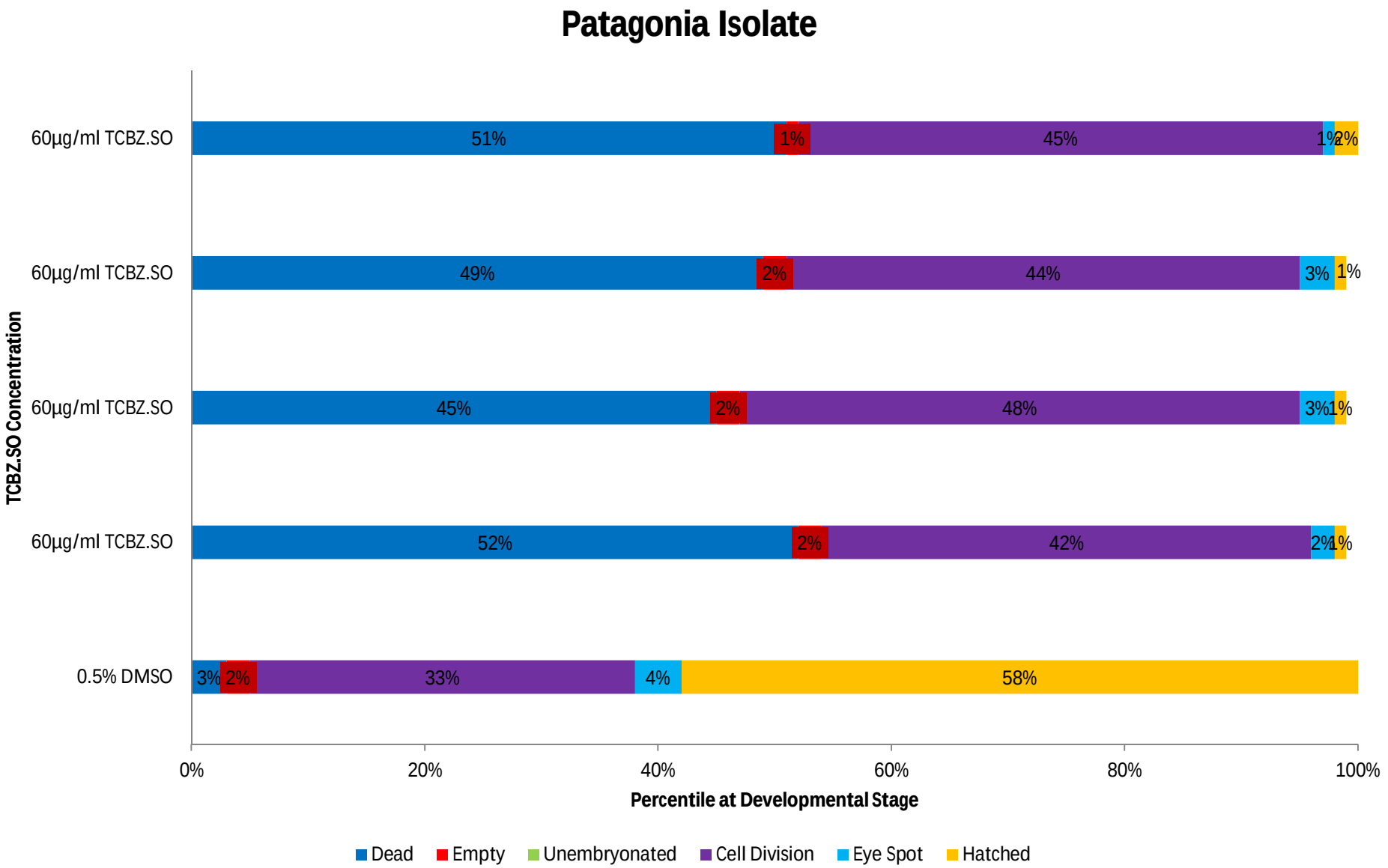


Fairweather et al. (2012). Vet. Parasitol. 183, 249-259.



Fairweather et al. (2012). Vet. Parasitol. 183, 249-259.

2-FARM SHEEP STUDY

- Pre- and post-treatment data from 2 farms.
- Sheep divided into groups of 10 and colour-coded.
- Individual and composite samples collected pre-treatment and 3 weeks post-treatment with TCBZ.
- Samples analysed “blind” for FEC and by CRT at 2 different centres [CRT at Belfast (VSD)].

		PRE-TREATMENT			POST-TREATMENT		
FARM	SHEEP GROUP	ELISA POOLED SAMPLE	NUMBER OF SHEEP ELISA-POSITIVE	EGGS per 50g	ELISA POOLED SAMPLE	NUMBER OF SHEEP ELISA-POSITIVE	EGGS per 50g
1	Green	1.068*	10/10	1080	0.221*	8/10	19
1	Yellow	1.382*	10/10	16	0.027	0/10	78
1	Red	0.630*	9/10	29	0.019	0/10	29
1	Orange	0.997*	10/10	2280	0.189*	5/10	16
1	Blue	1.283*	10/10	480	0.007	0/10	53
1	Purple	0.913*	10/10	1540	0.004	1/9	18
		(* = positive)	Total:59/60			Total:14/59	
2	Red	0.211*	4/10	390	0.040	0/10	76
2	Blue	0.450*	7/10	187	0.108	4/10	89
2	Orange	0.326*	7/10	600	0.059	1/9	22
2	Purple	0.108	2/10	39	0.105	1/10	235
2	Green	0.322*	6/10	465	0.035	1/9	141
		(* = positive)	Total:26/50			Total:7/48	

Interpretation of data

- Discrepancies between the results of the 2 tests.
- Results may illustrate the problems in determining whether there is resistance or not.
- Need to obtain corroborating evidence from more than one test to be confident of diagnosis.

Improved control of liver fluke infection on beef and dairy farms in the UK

Professor Diana Williams
School of Veterinary Science/Institute of Infection and Global Health, University of Liverpool

What I am going to say!

- Prevalence of infection
- Forecasting
- Drug resistance
- Treatment options in the face of TCBZ resistance

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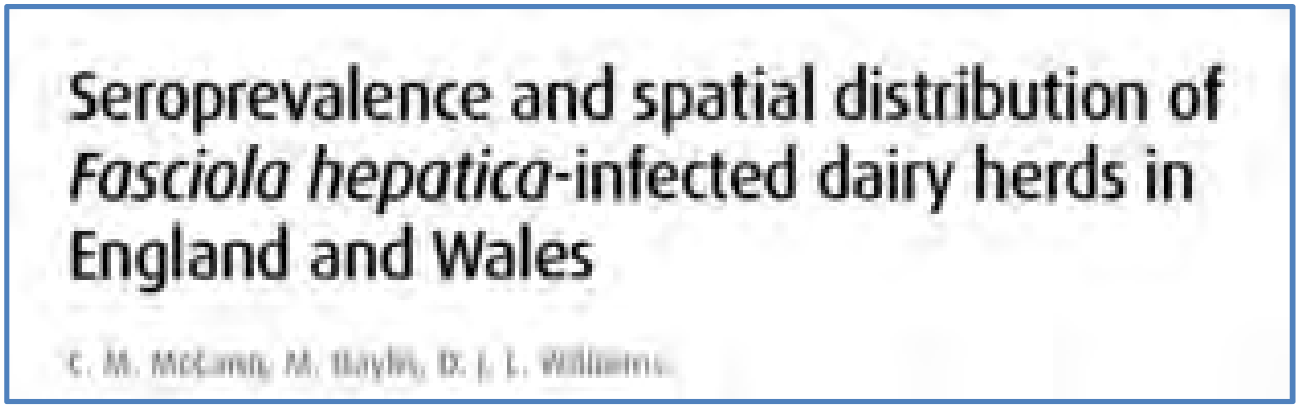
Spatial distribution of fluke in dairy herds in England and Wales

Bulk milk tank samples tested from 3,100 dairy herds


Seroprevalence and spatial distribution of *Fasciola hepatica*-infected dairy herds in England and Wales

C. M. McCann, M. Baylis, D. J. L. Williams

Veterinary Record 2010 166 612-617

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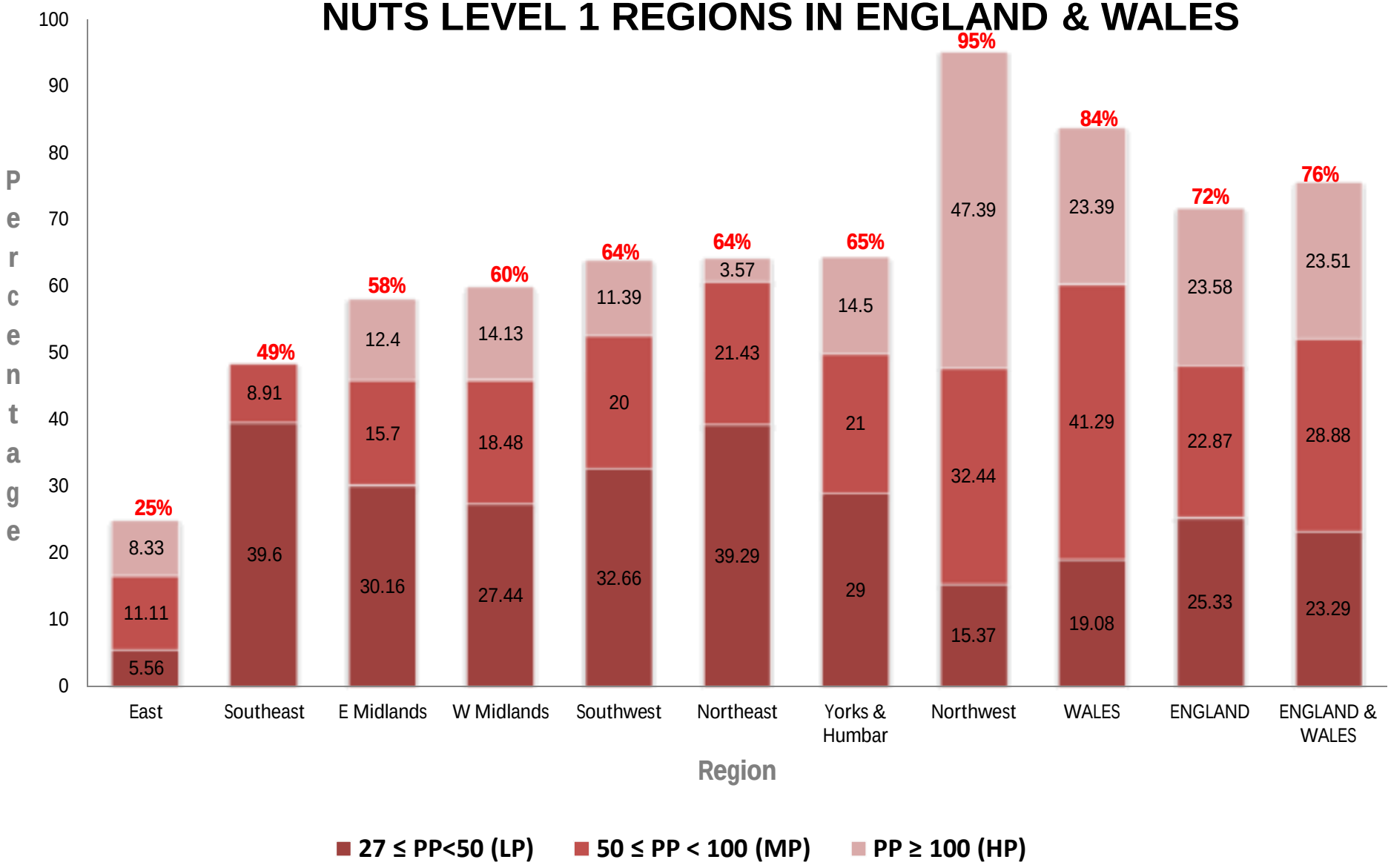
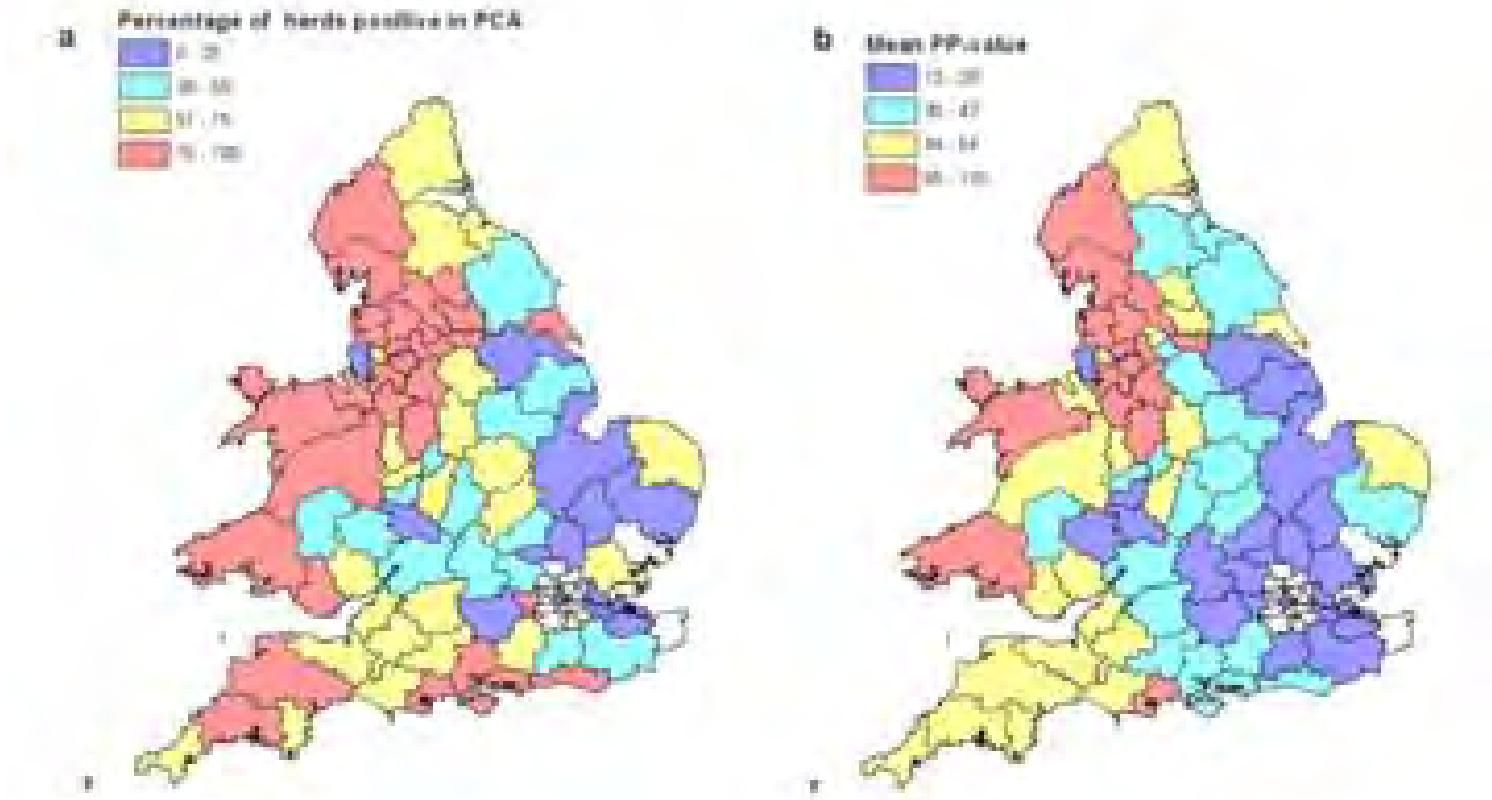
PREVALENCE AND DEGREE OF *F. HEPATICA* INFECTION IN NUTS LEVEL 1 REGIONS IN ENGLAND & WALES



Distribution of *F. hepatica* according to Post Code Area



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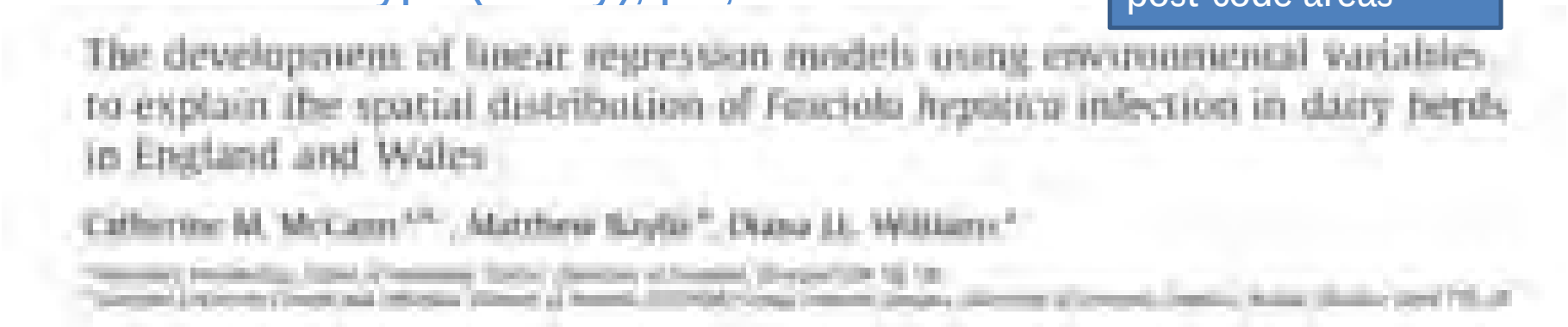
Predictors for distribution of fluke in the UK

- Rainfall
  - Summer and autumn
  - Previous five years
- Temperature
  - Warm winters
  - Cool summers
- Physical factors affecting snail populations
  - Altitude, slope
  - Soil type (sandy), pH, minerals

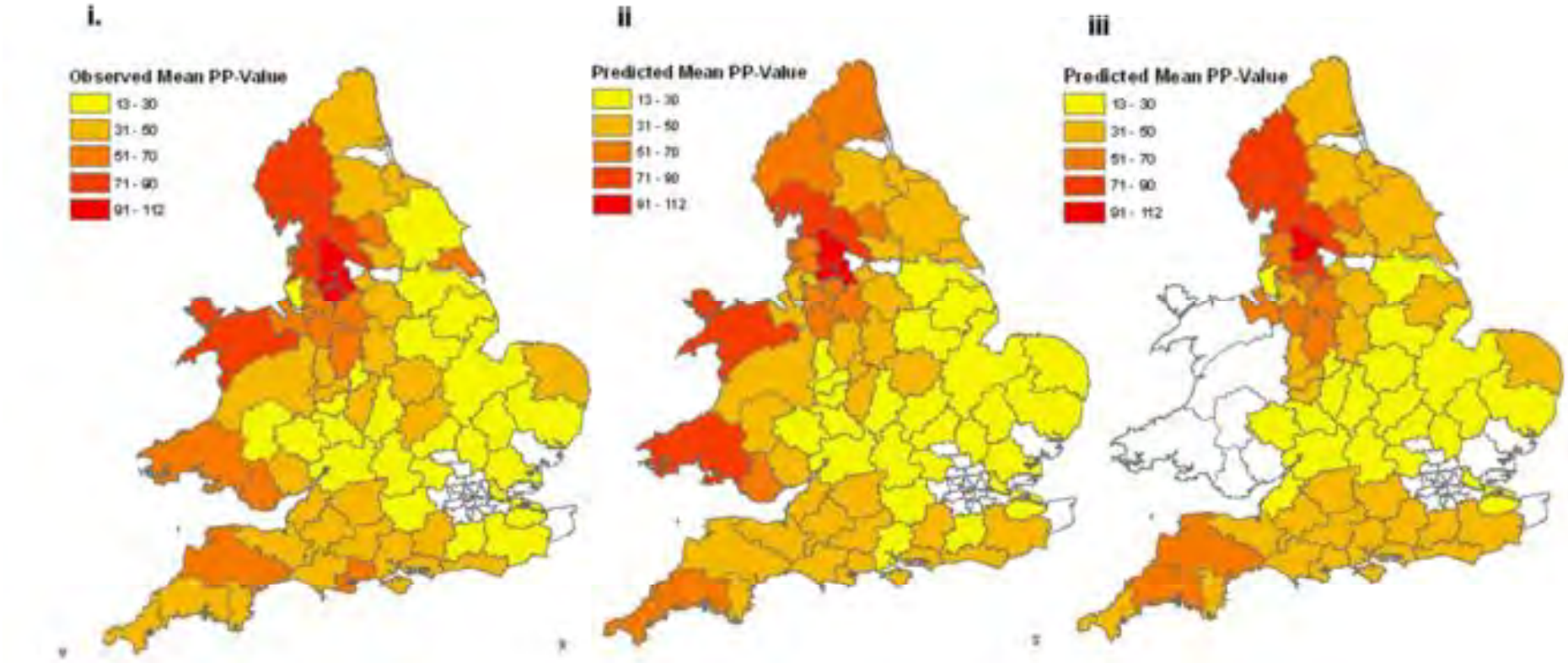
Models explain about 78% of variation between post-code areas



The development of linear regression models using environmental variables to explain the spatial distribution of *Fasciola hepatica* infection in dairy herds in England and Wales

International Journal for Parasitology 2010 40 1021-1028

Observed mean herd PP value for post code areas of England Wales and mean PP values for PCA predicted by logistic regression models 3 (ii) and Model 6 (iii)

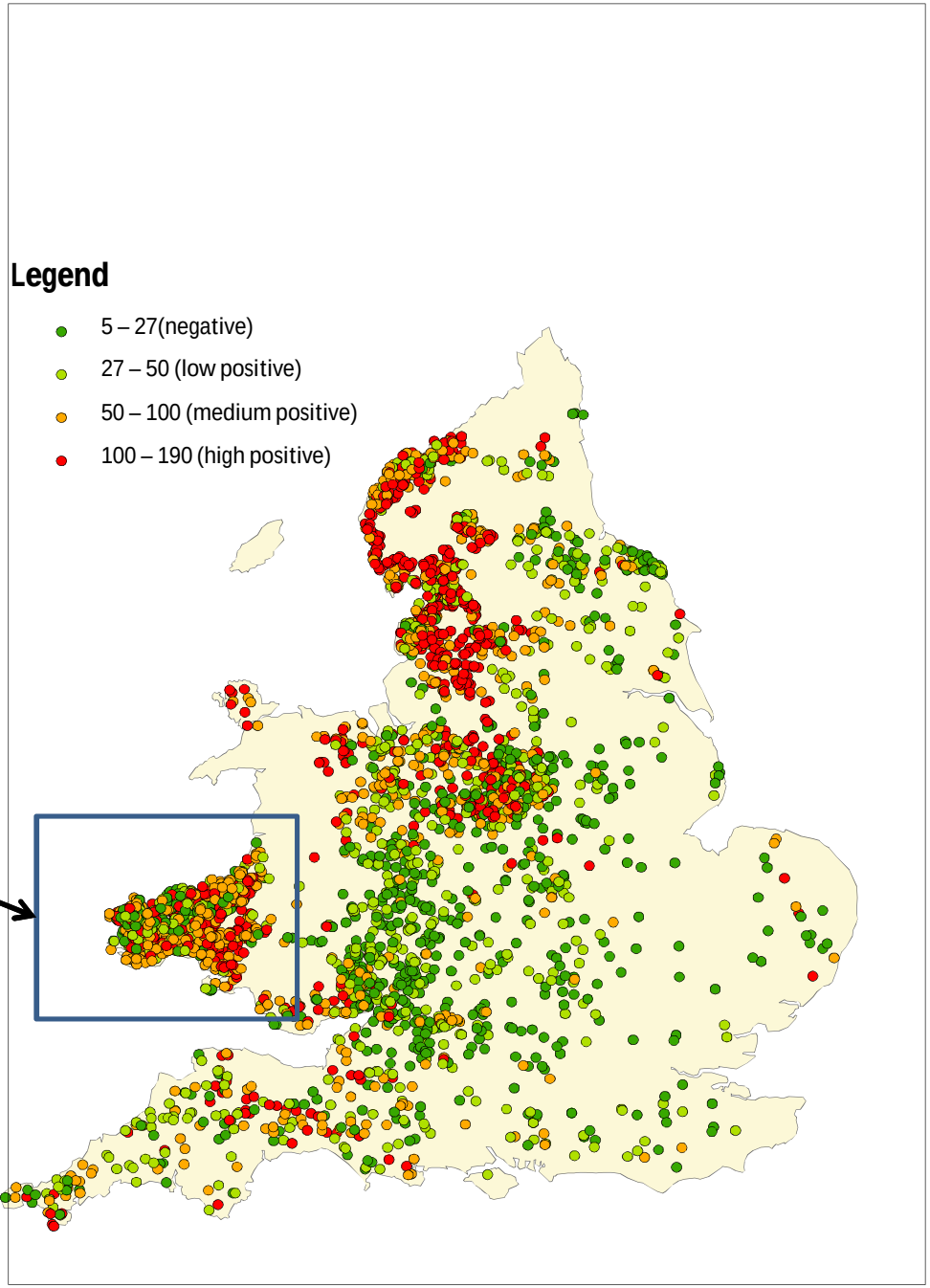


The predicted PP values were not significantly different from the actual values.

Farm specific factors determine fluke prevalence on a farm

Within one region, same climate but significant differences between neighbouring farms

Why?



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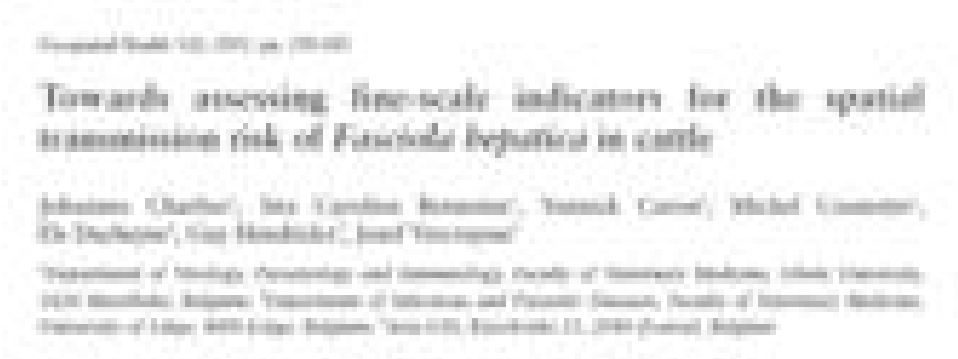
Farm specific factors

- Presence of snail habitat and snails
- Drainage of pasture
- Month of turnout
- Stocking rates
- Type of watering area
- Presence of sheep



Towards assessing fine-scale indicators for the spatial transmission risk of *Fasciola hepatica* in cattle

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Area of North Wales where study was conducted

36km^2 containing one farm per km^2 block. Sampling sites shown in red.

Questionaire used to identify risk factors and faecal samples collected from sheep or cattle on two occasions – feb, march 2012. Survey of snail habitats

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Univariates of ordinal logistic regression of actual FECs on 23 sheep farms

Variable	Coefficient	P value (≤0.1)	Odds Ratio	L-95% CI	U-95% CI
Drainage	1.94590	0.09	7	0.74	66.37
Silage	-2.38772	0.02	0.09	0.01	0.83
Number of Cattle	0.0324816	0.058	1.03	1	1.07
Manure	-2.38772	0.033	0.09	0.01	0.83
Combinex	1.84534	0.096	6.33	0.72	55.42
NDVI1*	0.0826876	0.073	1.09	0.99	1.19
NDVI2*	0.330307	0.081	1.39	0.96	2.02

*NDVI – normalised Difference Vegetation Index

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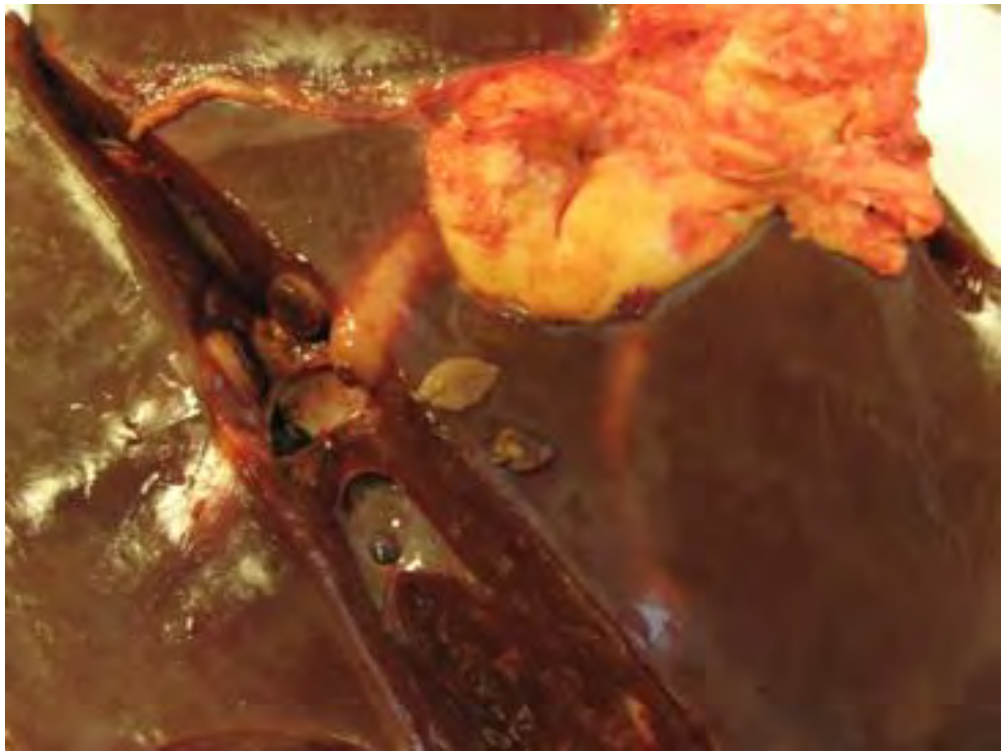
More work needed to identify management factors that affect prevalence of infection – reduce reliance on drugs



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There is increasing evidence of resistance to triclabendazole in UK

- In collaboration with the AHVLA, developed a faecal egg count reduction test, to evaluate triclabendazole (Fasinex) failure in the field
- Twenty five farms in Britain tested
- Evidence of drug failure on seven farms
- Six in Wales, one in Scotland

A composite faecal egg count reduction test to detect resistance to triclabendazole in *Fasciola hepatica*

FECRT using composite faecal samples

Farm	Pre-drenching faecal egg count	Post-drenching faecal egg count	Significant reduction in faecal egg count	Mean % reduction
2	335	116	-	65
5	1,681	563	-	66
6	6,545	9,502	-	0
7	3,099	5,547	-	0
8	46,389	29,161	-	37
22	455	182	-	60
25	9866	9890	-	0

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What do we do on farms with evidence of TCBZ resistance?

- Farm 1 – grazes 1600 ewes mainly on salt marshes but sheep brought onto freshwater flukey pasture for spring tides (~ 2 weeks).
- TCBZ resistance identified in 2009.
- Used closantel every 6-12 weeks to reduce pasture contamination for two years.
- Advised to switch to nitroxynil for 2011 – sheep died as a result of fluke in Oct/Nov, 2011
- Pre and post treatment fluke counts after closantel and nitroxynil, designed a more strategic control programme
- Select treatment according to the time of year – closantel during the high risk autumn period, nitroxynil or albendazole (ovidical) when predominantly adult flukes are present.

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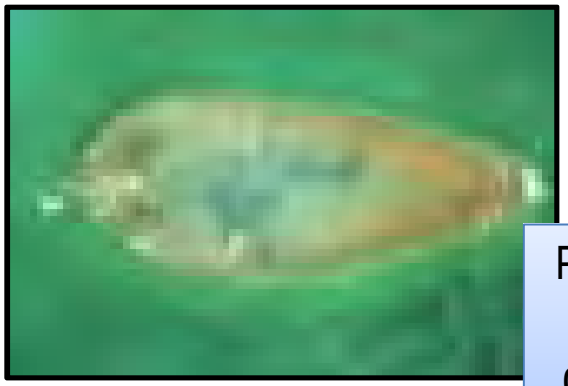
Cur arch ol

Immuno-modulation
UofL and BBSRC funded PhDs
Daphne Jackson Trust Fellow
[DJLW, MB, Grace Mulcahy]

Paravac – EU
Modelling vaccine

Tesco
Pilot project to investigate fluke on 700 Tesco dairy farms – improved control, forecasting, impact on concurrent disease and productivity
MB, GP, Rob Smith

Forecasting future risk of disease in Europe
MB, DJLW JvanDijk Roger Daniel



Mapping TCBZ resistance - BBSRC
Fluke genome, TCBZ resistance
JHodgkinson; SPaterson, JLaCourse; DJLW

PhD on fluke population genetics(JH; ; DJLW)

enor
ium

Prevalence of TCBZ resistance (JH; G Pinchbeck; DJLW, Roger Daniel

BBSRC
Improved, Sustainable Control of fasciolosis in dairy and beef cattle
UofL, MRI, CEH, SAC, AHVLA
EBLEX, QMS, HCC, Agrisearch, Dairyco
Sept 2012

Human fasciolosis – NTD (MRC/Gates)
JH
R Stothard, JlaC

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Acknowledgements

- Catherine McCann, Veterinary Parasitology
- Matthew Baylis, Liverpool University Climate and Infectious Diseases of Animals (LUCINDA)
- Jen Claridge, Veterinary Parasitology
- Peter Diggle, Lancaster/Liverpool Universities
- Jan van Dijk, Veterinary Clinical Sciences
- Roger Daniel, AHVLA
- Harry Swales and Jack Balkham
- Farmers and their families and their Vets
- Funding from the EU & BBSRC

Abattoir data collection/dissemination

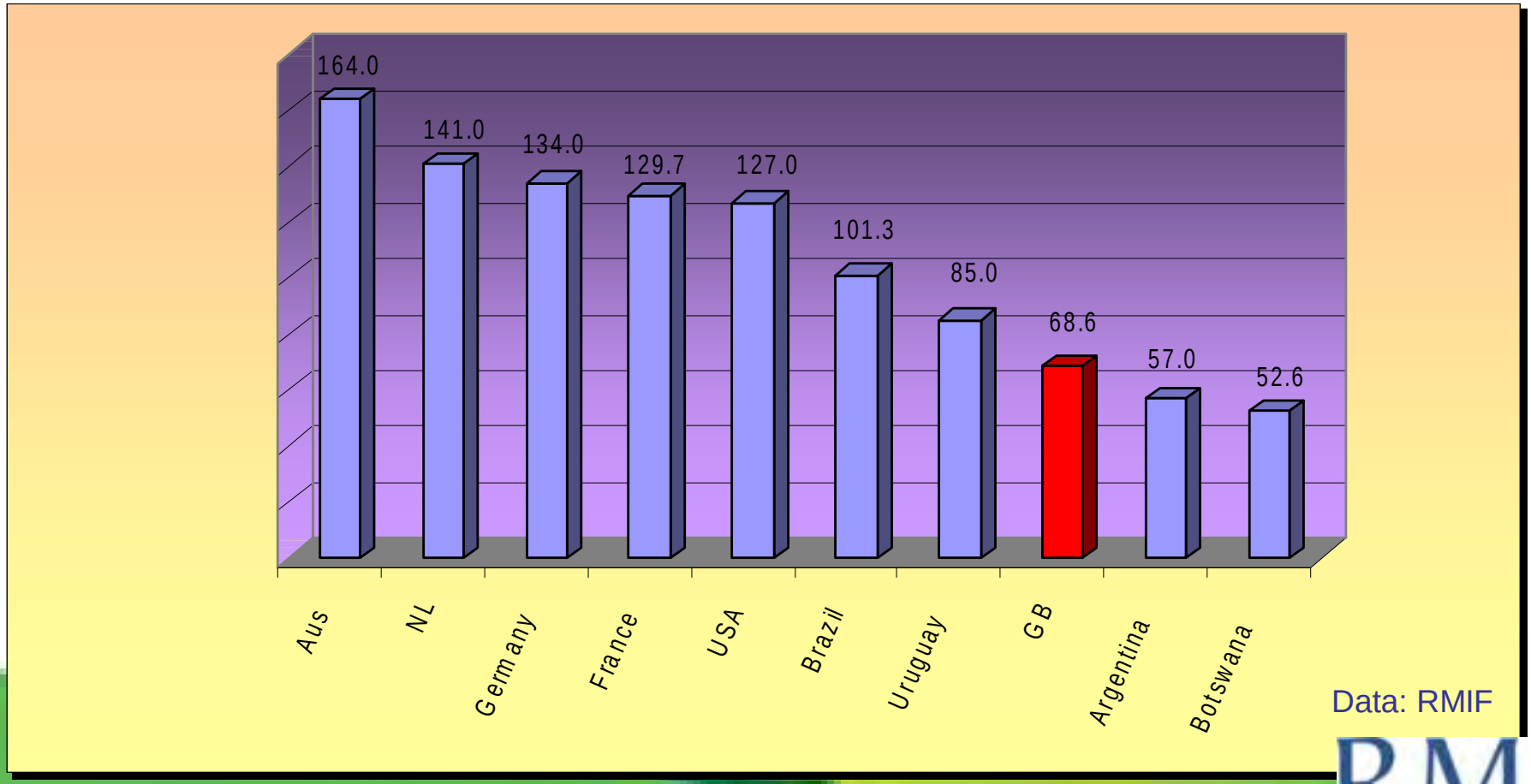
Phil Hadley

Phil.hadley@eblex.ahdb.org.uk

Background from a meat industry perspective

- Historically valuable covering the cost of killing with zero money changing hands for the process
- Disease resulted in immediate loss of overseas markets as well as home with little demand hence became a disposal cost/pet food commodity
- Opening export opportunities esp Asia mean focus is back on and need to upgrade facilities & skills to add value

Cattle fifth quarter in selected countries (US$)



Data: RMIF

International comparison

- The difference between Dutch and British fifth quarter value is £ 36.5 / head
- This means a theoretical loss of value to the UK beef sector of £ 96.5 M
- Poor perception of offals in UK-processor, MHS & consumer

Size/value of the domestic market

- Domestic offal consumption up 67% from 2003 to 2008 with a market value of £62M (Mintel 2009)
- AHDB: consumption stable at 12,500T and market worth £30M. Liver £22M. (AHDB 52wks April 2012)
- 2008-2010: A £2.2M cost of disposal turned into an income of £13.3M- a £15.5M turnaround following a project by MLCSL Ltd (QMS Annual review 2009/10)
- Export opportunities? More products?

Abattoir data-questions?

- What information is collected
- Who by
- Who ‘owns’ it
- How is it communicated
- What's the value of this
- Where do the responsibilities lie

5.58M ruminant cases ’08-11.

Losses

Species	Numbers lost in 2011	Value £ (difference between marketplace and pet food realisation)
Cattle (fluke)	510,269 (22%)	£2,296,211
Sheep (fluke and tenuicollis)	828,980 (6%)	£646,612

Add in productivity loss (1kg wk), mortality, welfare etc and the impact is huge (est £25-30 per cattle case (£12.7-15.3M))

Beef cattle (ADAS 2012)

Treatment & Cost	£/head
80 days longer on farm @ £1.80 day/hd (variable costs)	144
10% reduction in carcass value	79
Costs of additional finance for 80 days (2% over base)	6
Total loss/hd	229
Treatment + labour	3
Cost benefit/hd	226

Sheep (ADAS 2012)

Treatment & cost	£/lamb
30% reduction DLWG (4wks extra @ £0.90 grass keep/wk + 5kg conc/wk @ £200T	4
Cost of treatment + labour	0.44
Cost benefit/hd	£3.56

Rejections and loss

- A lot of material, particularly livers, is lost at meat inspection due to parasitic infections
- Also material is lost or devalued due to poor practice- gall bladder removal, pluck removal etc
- It’s the losses associated with farm practice that we need to work harder on

Messages

- Need to get rejection messages to producers to drive change
- Currently ad hoc
- Difficulties in collecting and collating the data
- Maybe FCI and new recording methods by FSA will make this less challenging?
- BPHS as a model?

Communication

- Requirement for producers to provide Food Chain Information to abattoirs with ruminant stock (Jan 2010)
- Minimum elements approach to improve food safety, animal health and welfare
- Collection & communication of Inspection Results (CCIR) requirement offers an opportunity
- FSA commissioned study to investigate

FSA study objectives

- Review relevance of current system against objective- (improving health/welfare)
- How FCI/CCIR is used by FBOs, OVs and producers/vets
- Improvement gaps for production or official control needs

Feedback mechanisms

- FSA Innova system- on-line touch screen terminal for recording inspection results
- Handful of plants receiving data this way along with a small number of producers
- Trials for late 2012
- Should lead to greater industry uptake and communication through to producers
- 70% of producers who received feedback responded to it

- Feedback and who advises? Calls to abattoir for support
- Producer response to fluke unlikely to involve vet advise- is this an issue? Vast majority did not want vet to also receive info
- Where does the supporting information come from and is there a need for concerted effort
- IT compatibility in plant needs to be overcome

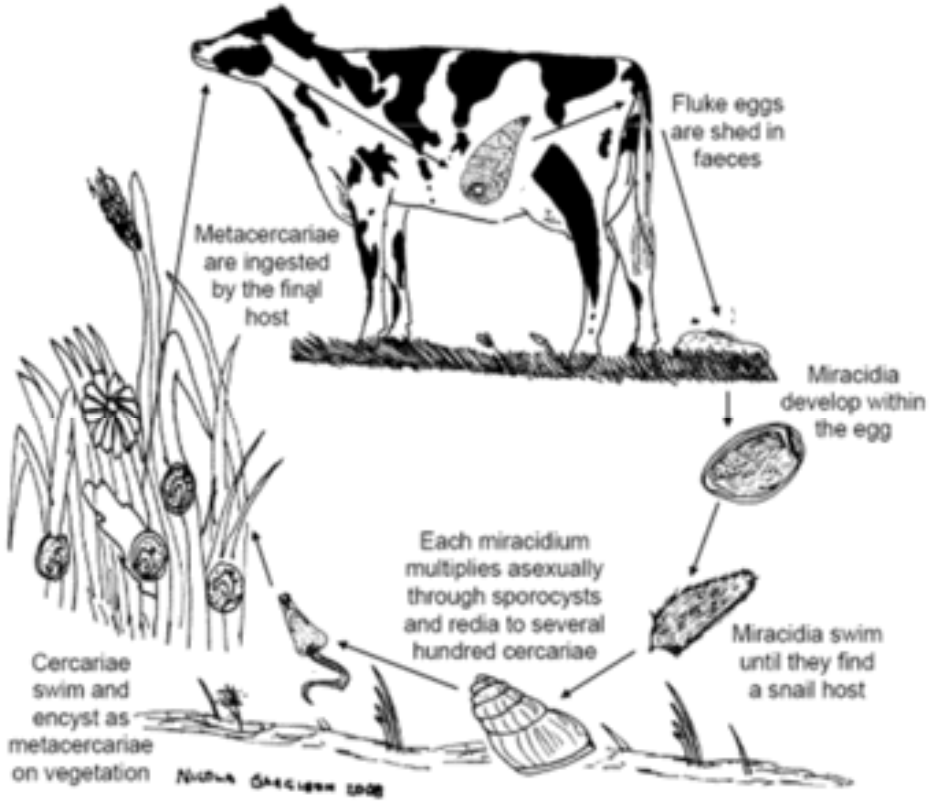
Opportunities

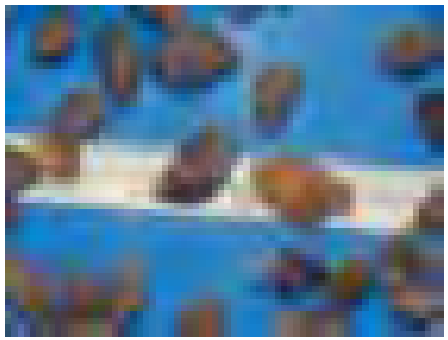
- Provision of liver data:
  - Identify fluke presence
  - Monitor success of control and strategy
  - Associate value in terms of production/loss
  - Set KPIs
  - Allow benchmarking internally and externally
  - Identify trends
  - Improve productivity/reduce losses

Paramphistomum cervi?

- Primarily parasites of wild and farmed deer, but also seen in cattle (and sheep)
- Limited or unknown clinical significance
- Similar free-living stage lifecycle to *F. hepatica*
- Lifecycle and pathology in the ruminant host

.....



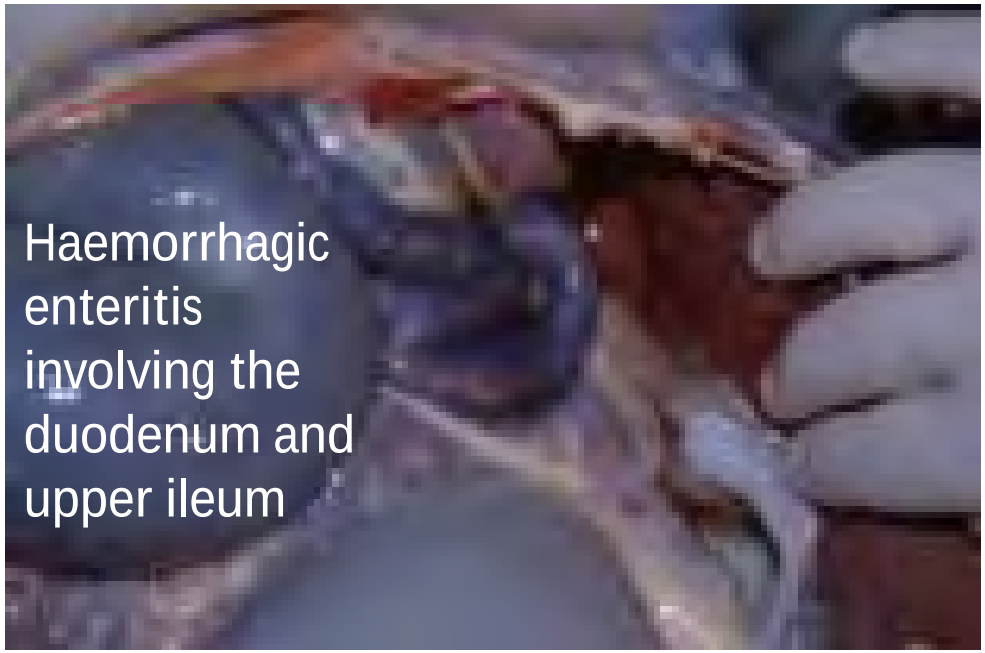


Planorbis spp. and *Bulinus* spp. water snails? Other snails?

Pathology

- Metacercariae excyst in the duodenum.
- Immature flukes attach and ‘plug’ feed for about 6 weeks.
- Young flukes migrate to the forestomach where they mature.
- Adult flukes attach to the forestomach wall using their sucker and feed on blood.

Pathogenesis is associated with the intestinal and migratory phases.



Calicophorum daubneyi

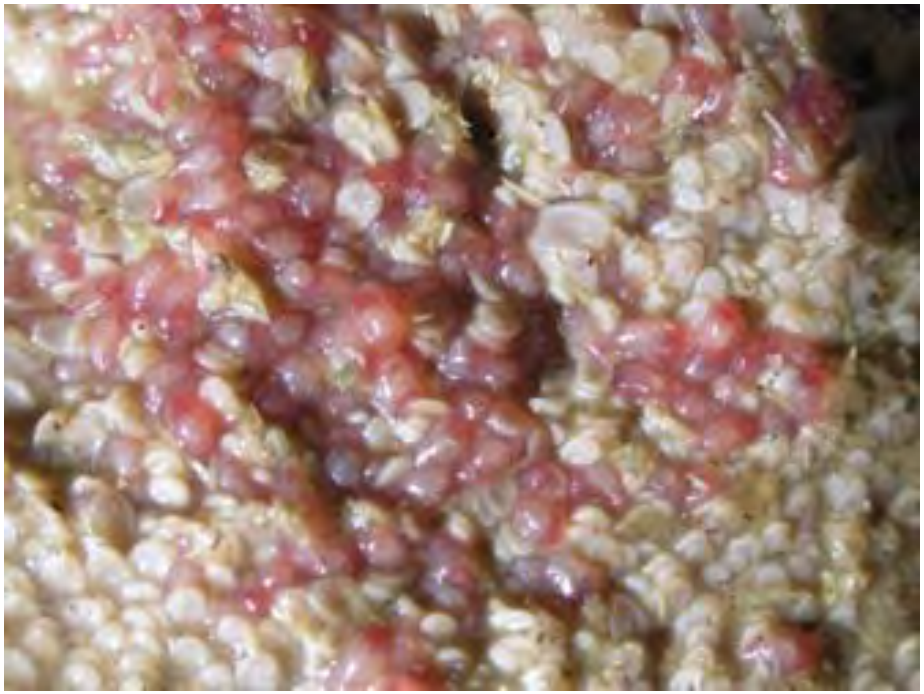
Paramphistomum cervi

F. heptica

Image courtesy of Sian Mitchell, VLA Carmarthen

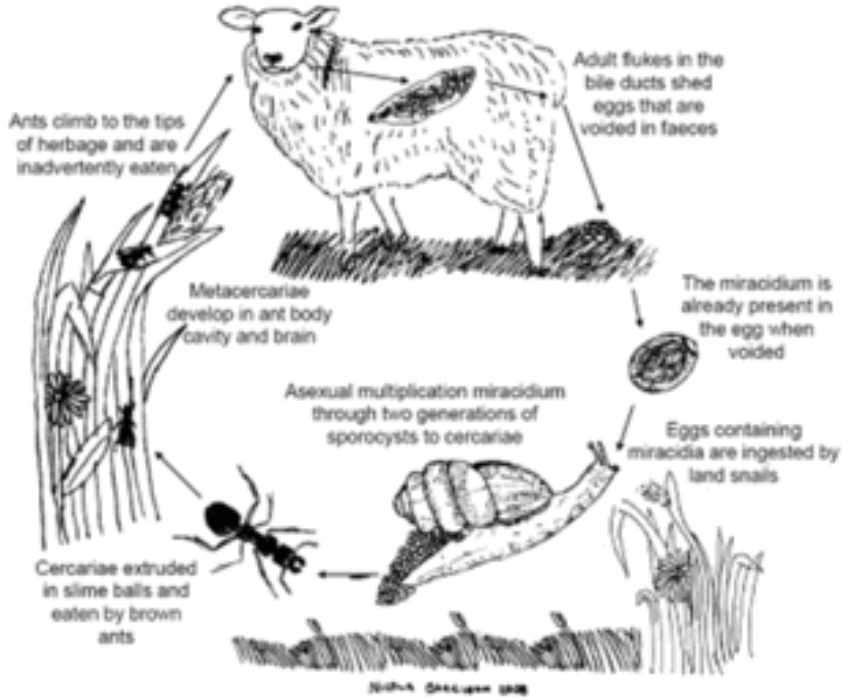
Treatment of paramphistomes.

- Oxyclozanide
  - adults only



Dicrocoelium dendriticum

- **Eggs** containing **miracidia** hatch after ingestion by land snails.
- Two generations of **sporocysts** in the snails.
- **Cercariae** are extruded in slime that adheres to vegetation
  - slime balls are ingested by ants.
- **Metacercariae** develop in ant body cavity (and brain)
  - brain lesions induce ants to climb to tip of herbage.
  - ants are ingested by ruminants.
- Metacercariae hatch in small intestine.
- Immature flukes migrate along the main bile duct to the liver
  - no parenchymal migration.
- **Adult flukes** in bile ducts survive many years.
  - significant pathology and clinical signs only in heavy infections
  - slaughterhouse condemnation of livers

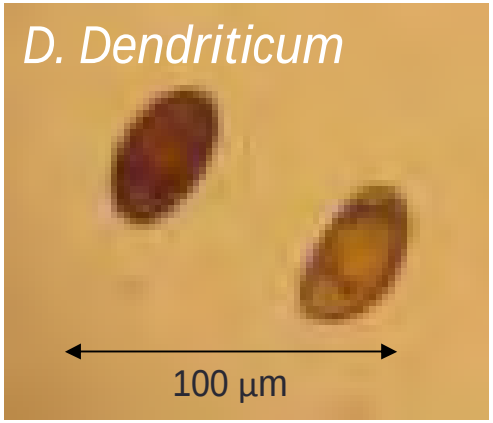


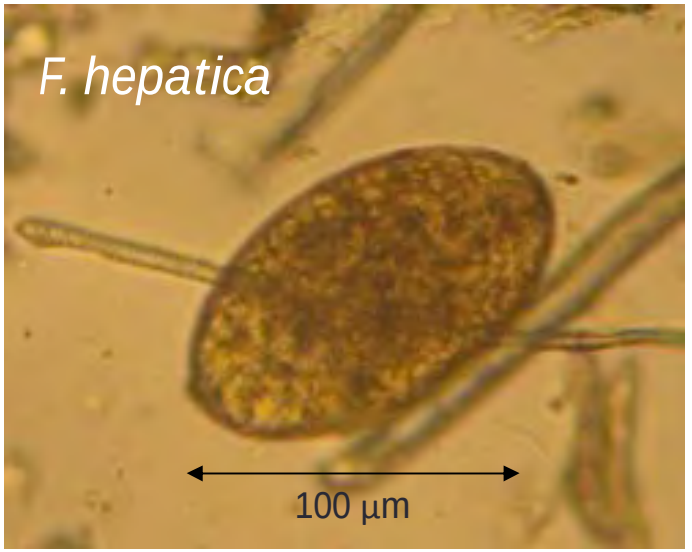
Formica spp.
ants

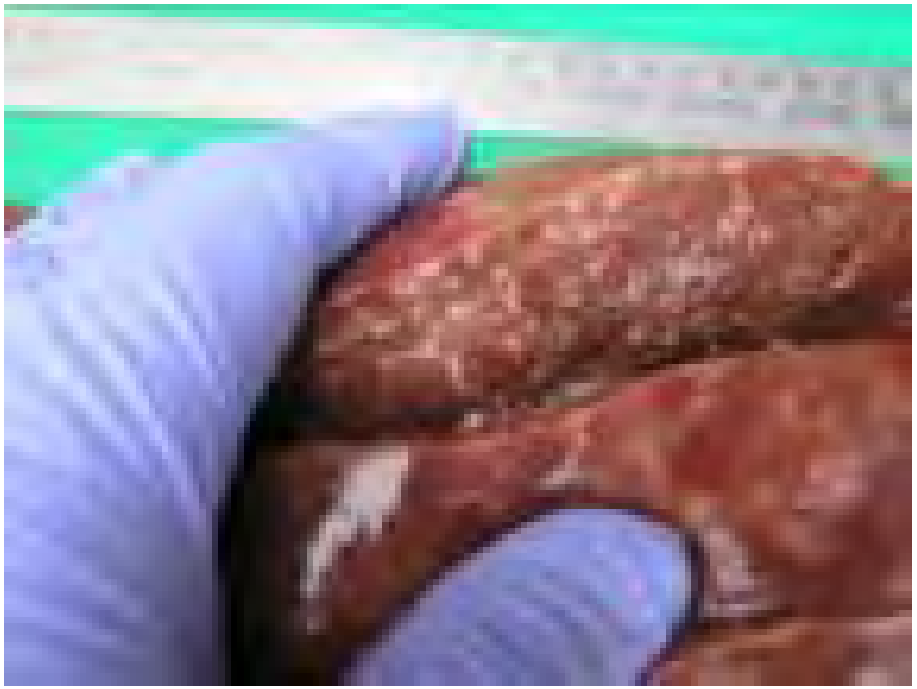
Cercaria

Helicella itala
land snails

Dicrocoelium dendriticum







Example

Investigation of ill thrift and photosensitisation

Isle of Coll

Example

Investigation of ill thrift and photosensitisation

- July 2011
- Ill thrift in sheep flock
  - (~250 ewes, 250 lambs and 65 ewe hoggs)
- 32 ewe hoggs with photosensitisation
- Cattle unaffected
  - (~30 cows, 30 calves and 12 heifers)
- ~1000 acres (414 ha) of machair at the west of the island

Example

Investigation of ill thrift and photosensitisation

Mean (n=10) epg	Trichostrongyle	*Dicrocoelium dendriticum*	*Fasciola*
Ewes	82	45	0
Lambs	88	6	0
Hoggs	114	909	0
Cows	0	150	0

Example

Investigation of ill thrift and photosensitisation

Mean (n=10) epg	*Dicrocoelium dendriticum*
Ewes	45
Lambs	6
Hoggs	909
Cows	150

- How can the *D. dendriticum* egg counts be interpreted?
- How has the problem arisen?
- How can the immediate problem be managed?
- How can dicrocoeliosis be controlled on this farm?

Example

Investigation of ill thrift and photosensitisation

Machair

Example

Investigation of ill thrift and photosensitisation

Treatment of *D. dendriticum*

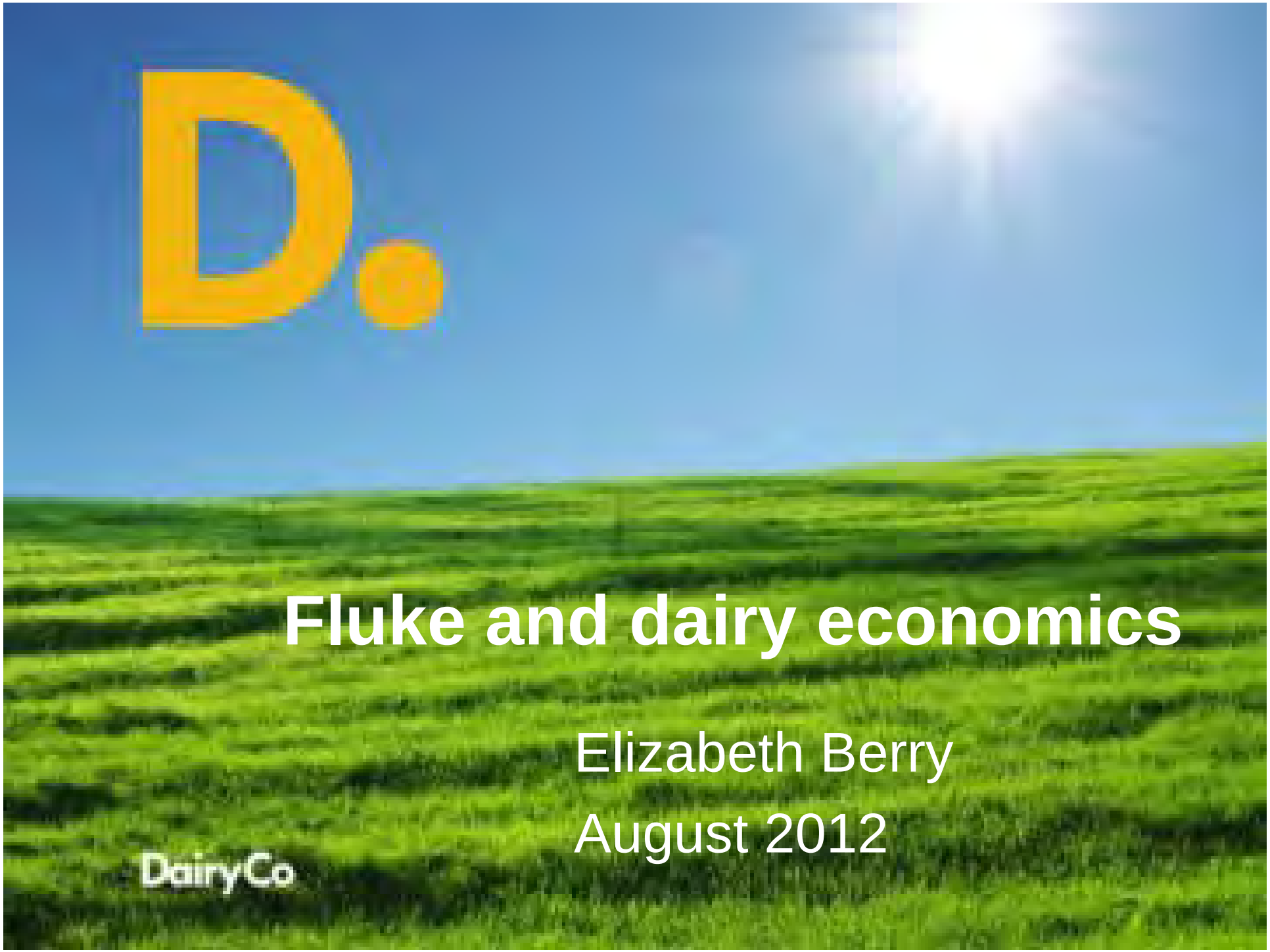
- some benzimidazole anthelmintics at high dose rates
  - netobimin
  - albendazole (15 mg/kg)
  - fenbendazole (15 mg/kg)

Example

Investigation of ill thrift and photosensitisation caused by *D. dendriticum*

D. dendriticum	11-7-11	2-8-11
Mean (n=10)	909 epg	189 epg
SEM	±115 epg	±45 epg
Reduction (%)		**79**
Cis (%)		88, 64

- Treatment with 15 mg/kg albendazole on 11th July 2011
  - resistance?
  - inherent lack of efficacy?
  - pharmacokinetics?
- How can the problem be managed?



Production losses

- Defra GHG mitigation – production losses
- Fluke in top 10 of diseases

- Decreased milk yield
- Reduced weight gain
- Reduced fertility
- Immuno-competence – liver compromised

Production losses

- Herd 1.5kg/cow/day ave 30kg)
- Individual 2kg/cow/day (ave 31kg)
  - Vet Parasitology 2011 180 237-242 Association between anti-F.hepatica AB levels in milk and production losses in dairy cows
- 0.7kg/cow/day (ave 23kg)+ longer inter calving intervals
  - Prev Vet Med 2007 78 57-66 Associations between Anti-F.hepatica Abl evels in bulk -tank milk samples and production parameters in dairy herds
- 299 euros/cow (yield and reproduction)
  - Vet Rec 2005 157 188-193 Estimating the financial losses due to bovine fasciolosis in Swtizerland

Treatment costs

- Albendazole (adult fluke)

  Milk withold for 60 hours
- Triclabendazole (one product)

  Start of dry period only

GB costs?

- 13,500 million litres – annual production
- 1.5 million dairy cows
- Percentage affected ?

But for 10% cows with a 10% loss in yield =150 million litres

- Average milk price 26 pence = 39 million
- Probably an underestimate!

Moredun Update – experiences with the Bio-X coproantigen ELISA

Philip Skuce, Danielle Gordon, Neil Sargison R(D)SVS & Ruth Zadoks,
Moredun Research Institute, Edinburgh

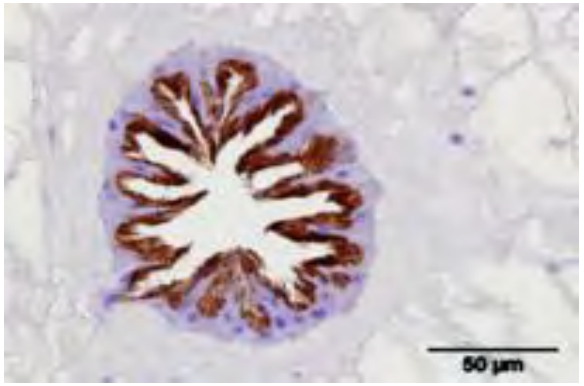
philip.skuce@moredun.ac.uk

SCOPS Fluke Workshop
8th-9th August, 2012

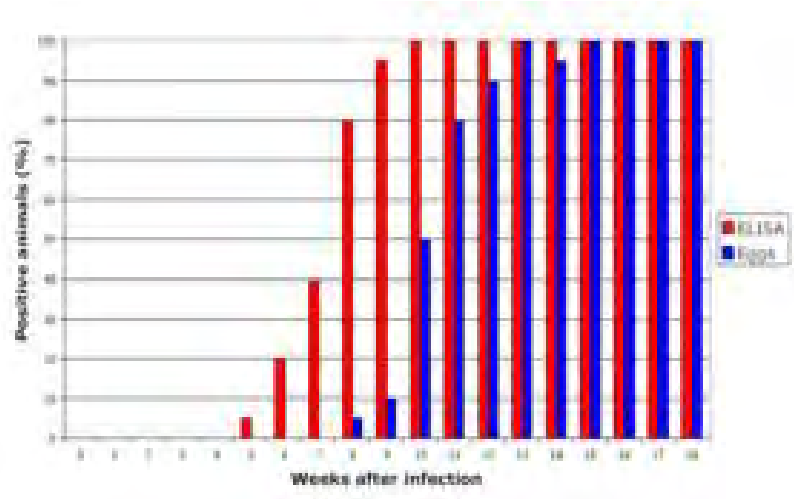
Bio-X cAgELISA (BIO K201)

- Based on MM3 MAb (Mezo et al, 2004), commercialised by Bio-X Diagnostics, Belgium
- Detects tiny amounts of fluke secretions in host faeces
- Non-invasive, convenient, potential for high-throughput etc.?
- Claimed to:

1. detect juvenile & adult fluke
2. indicate fluke burden
3. test –ve following successful treatment

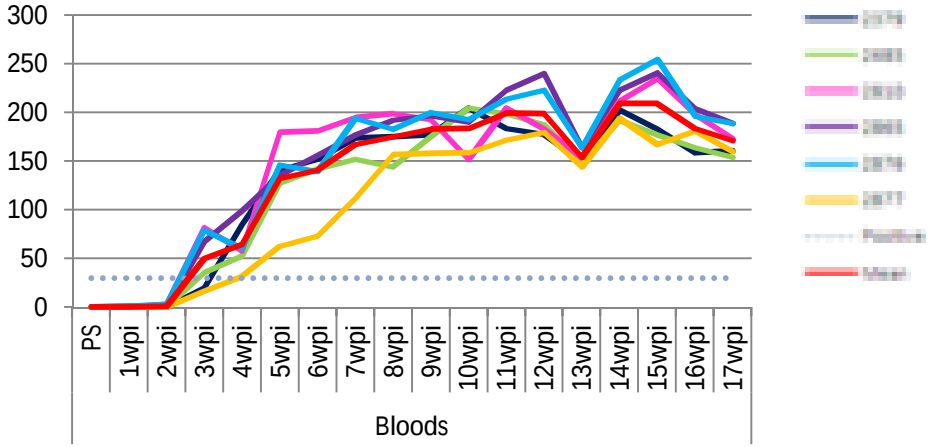


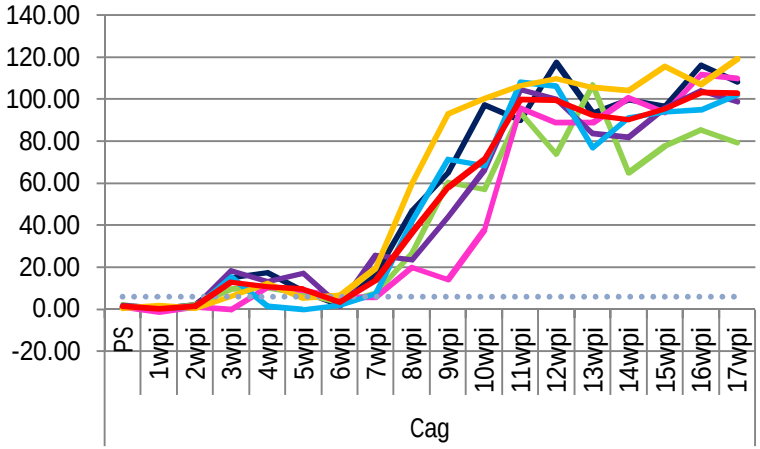
- Claims based on experimental infections

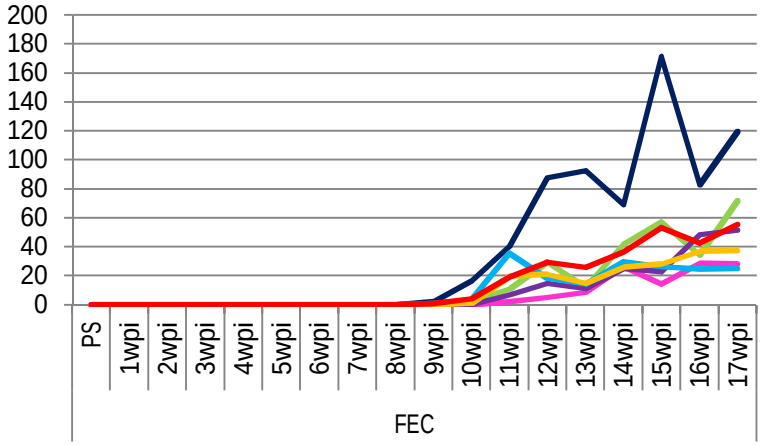


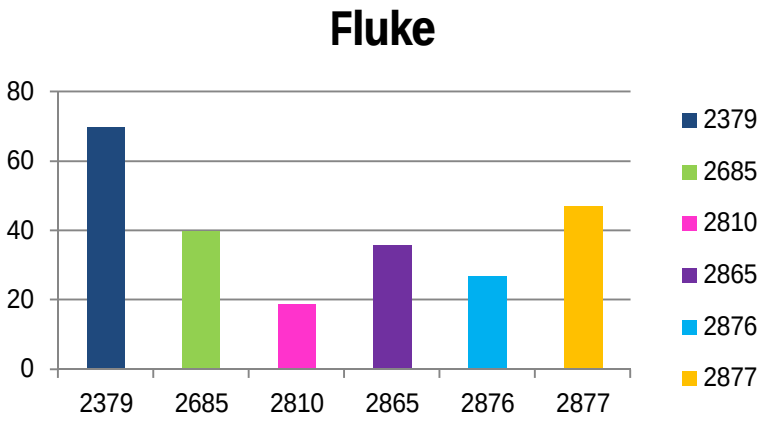
In-house evaluation: Exptal challenge model

6 male lambs, 1 year old, given ~150 *F. hepatica* cysts each, monitored for 17 weeks by FEC, cAgELISA and AbELISA:

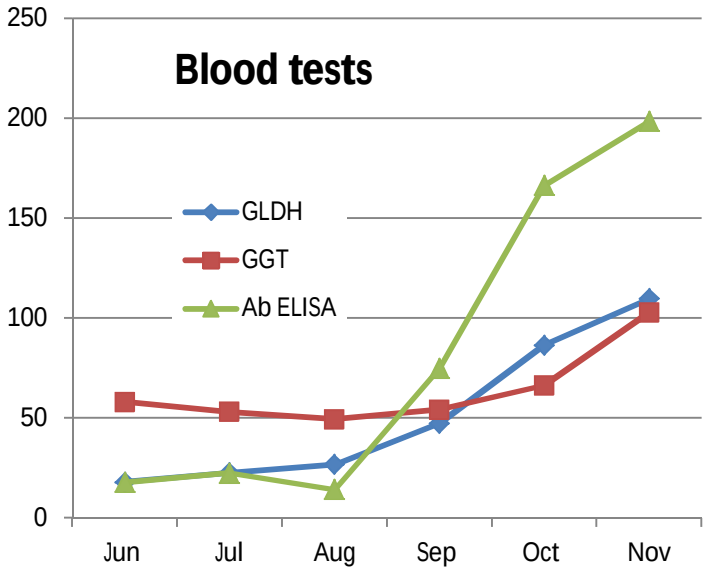


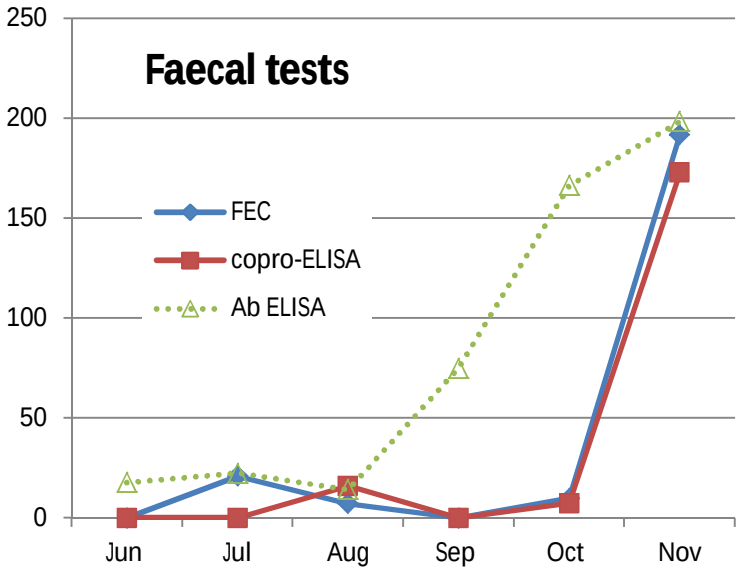






Field evaluation of cAgELISA



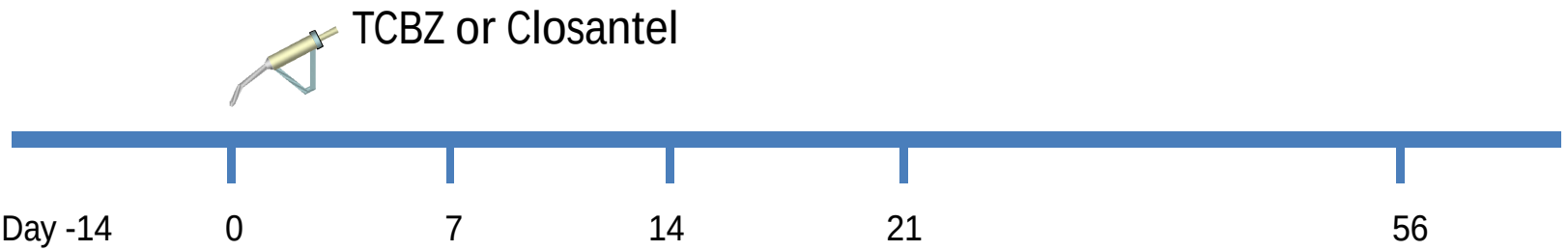


Results (n=26)

- Liver/bile duct enzymes always high in lambs, not a good indicator on their own
- Serum Ab ELISA first to become positive, useful if this year's animals
- cAgELISA at least as good/no better(?) than FEC but easier/faster to run in the lab

Treatment trial 2011 : Conducted on both farms in peak fluke season (Jan-March) ; Farm 1 housed, Farm 2 out-wintered:

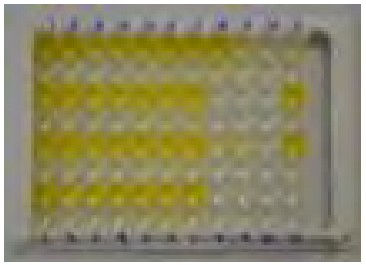
2 x groups of ~25 ewes on each farm (n=100):



Study design

- Pre-screen on Day -14 to ensure all ewes fluke +ve
- Treat on Day 0 with either TCBZ or Closantel
- Monitor by FEC and cAgELISA (= FECRT v CRT*)

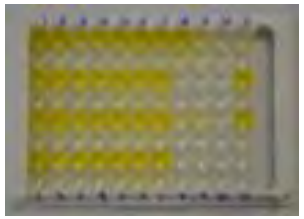
*Flanagan et al (2011) propose CRT based on 100% animals testing –ve d14 post-Tx

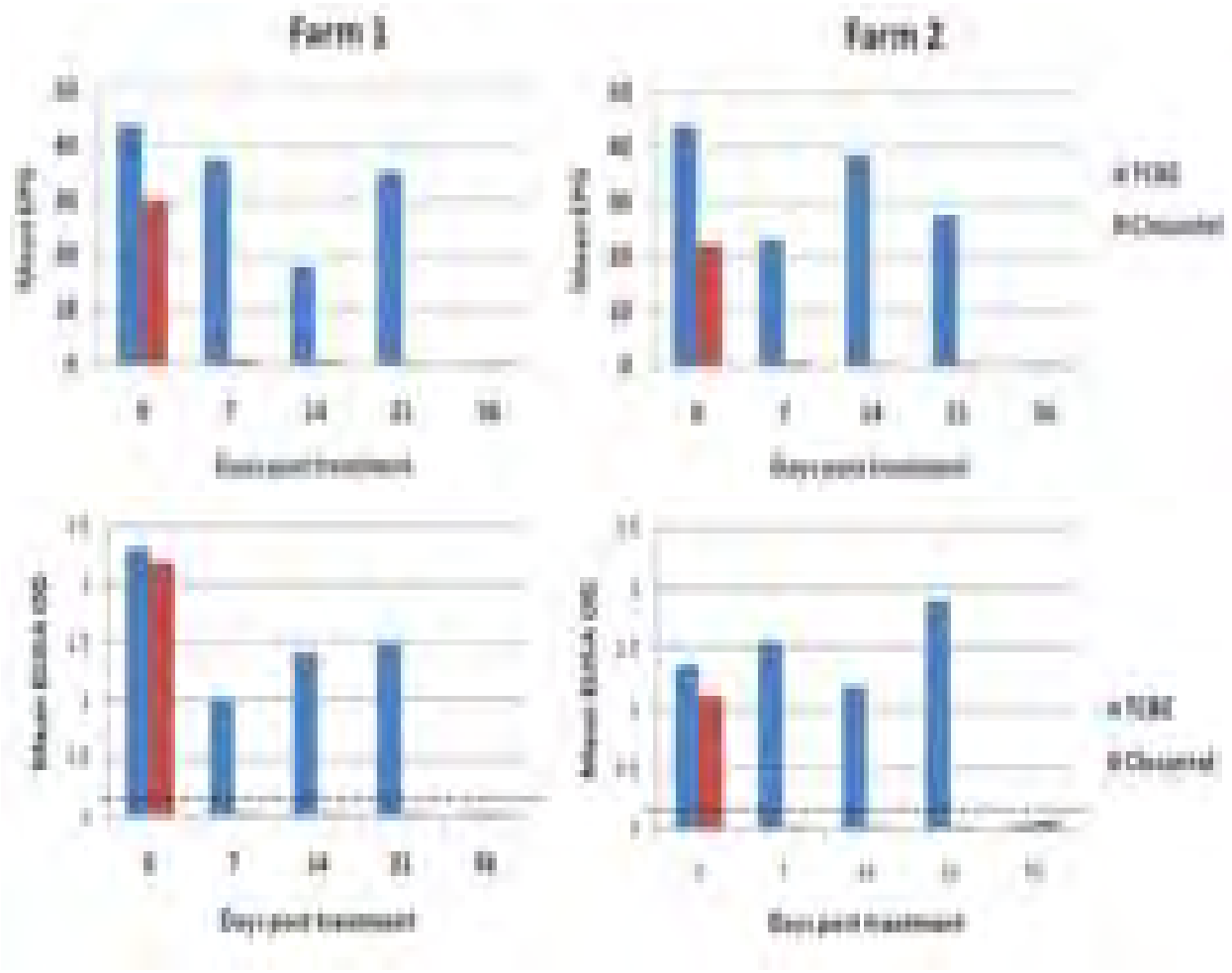


Outcome

FECRT

CRT





- Results comparable but FECs more variable group-to-group
- Some animals still shedding eggs after effective treatment, CRT clearly -ve
- Bring decision forward to d7 (FEC & cAgELISA)?

Gordon et al, 2012, Vet Para

Field Evaluation cont.

Postal survey 2011-2012 - Could we make faecal test(s) more practical for routine use on farms:

- Use a **composite** (i.e. group) sampling strategy rather than sampling individual animals (*cf* Daniel et al, 2012 Vet Record)

- Samples to be collected by **farmers** on Day 0 and Day 21 post-treatment

- ~35 sampling kits sent to sheep farms across UK, 20 provided appropriate pre- and post-treatment samples

Results

Range of infection levels & treatment outcomes; TCBZ successes & failures

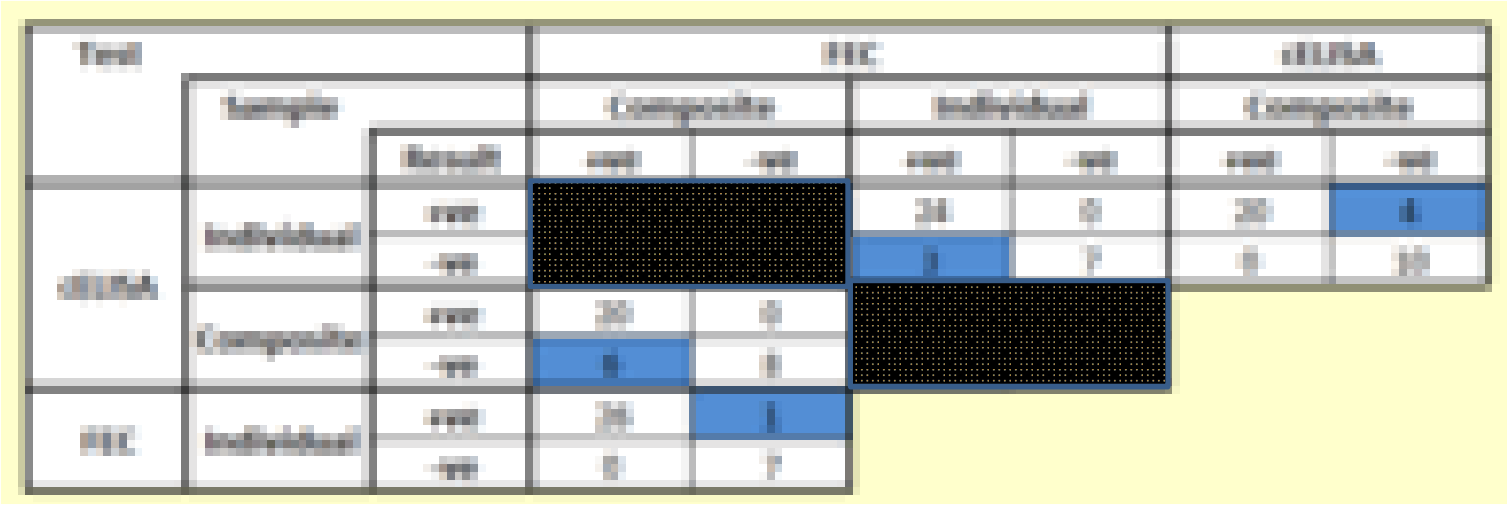
- No fluke eggs day 0 (no need to treat?)
- High fluke egg count day 0; no fluke eggs post-treatment
- High fluke egg count day 0; increased count post-treatment
- Paramphistomes (rumen fluke) detected on 2 farms *

* Rumen fluke persisting post-*Fasciola* Tx did not appear to interfere with cAgELISA

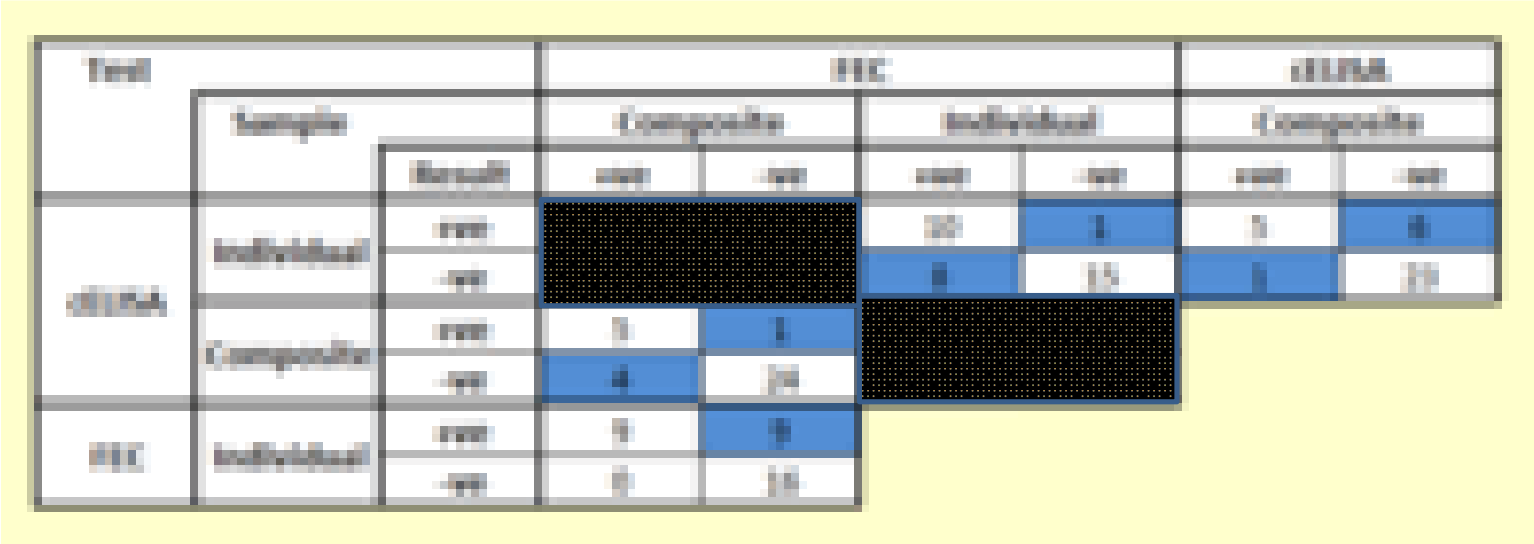
Preliminary data analysis

n=340 i.e. 34 groups of 10 sheep

Pre-Tx



Post-Tx



Breakdown of results – Pre-Treatment

1.		Composite FEC	
		+ve	-ve
Individual FEC	+ve	26	1
	-ve	0	7

- **Agreement:** 33 of 34 (97%)
- **Disagreement:** individual FEC +ve, composite FEC -ve
- **Interpretation:** "some loss of sensitivity with pooling"

3.		Composite cAgELISA	
		+ve	-ve
Individual cAgELISA	+ve	20	4
	-ve	0	10

- **Agreement:** 30 of 34 (88%)
- **Disagreement:** individual cAgELISA +ve, composite cAgELISA -ve
- **Interpretation:** "some loss of sensitivity with pooling & loss worse for cAgELISA than for FEC"

2.		Individual cAgELISA	
		+ve	-ve
Individual FEC	+ve	24	3
	-ve	0	7

- **Agreement:** 31 of 34 (92%)
- **Disagreement:** individual FEC +ve, individual cAgELISA -ve
- **Interpretation*:** "cAgELISA less sensitive than FEC"

4.		Composite cAgELISA	
		+ve	-ve
Composite FEC	+ve	20	6
	-ve	0	8

- **Agreement:** 28 of 34 (82%)
- **Disagreement:** composite FEC +ve, composite cAgELISA -ve
- **Interpretation:** "composite FEC more sensitive than composite cAgELISA"

* Buscher *et al*, 2011, Vet Para; FEC detected 17.8% of individuals on 64.5% of farms, *cf* 13.4% on 54.8% of farms by cAgELISA

Breakdown of results – Post-Treatment

1.		Composite FEC	
		+ve	-ve
Individual FEC	+ve	9	9
	-ve	0	16

- **Agreement:** 25 of 34 (74%)
- **Disagreement:** individual FEC+ve, composite FEC-ve
- **Interpretation:** "does composite sampling over-estimate Tx success?" (low numbers of eggs?)

3.		Composite cAgELISA	
		+ve	-ve
Individual cAgELISA	+ve	5	6
	-ve	1	23

- **Agreement:** 28 of 34 (82%)
- **Disagreement:** individual cAgELISA +ve, composite cAgELISA –ve
- **Interpretation:** "still loss of sensitivity with pooling"

2.		Individual cAgELISA	
		+ve	-ve
Individual FEC	+ve	10	8
	-ve	1	15

- **Agreement:** 25 of 34 (74%)
- **Disagreement:** individual FEC +ve, individual cAgELISA -ve
- **Interpretation:** "false +ve FEC post-Tx?" (sporadic egg release from gall bladder?)

4.		Composite cAgELISA	
		+ve	-ve
Composite FEC	+ve	5	4
	-ve	1	24

- **Agreement:** 29 of 34 (85%)
- **Disagreement:** composite FEC +ve, composite cAgELISA –ve
- **Interpretation:** "false +ve with composite FEC and/or false –ve with cAgELISA?"

Take home messages (sheep)

- cAgELISA imperfect test e.g. natural *vs* experimental infections, low vs high burden, trickle challenge etc.
- Individual cAgELISA good for determining Tx success in flocks with high fluke burden
- cAgELISA < FEC, whether individual or pooled and composite cAgELISA < individual cAgELISA
- Some unexplained anomalies - result of farm-specific factors?
- Criteria for CRT – minimum reduction threshold & minimum sample size *cf* 100% cAgELISA –ve d14 post-Tx?
- Hesitant to recommend widespread deployment of cAgELISA in the field until these issues are resolved

Evaluation of cAgELISA in cattle

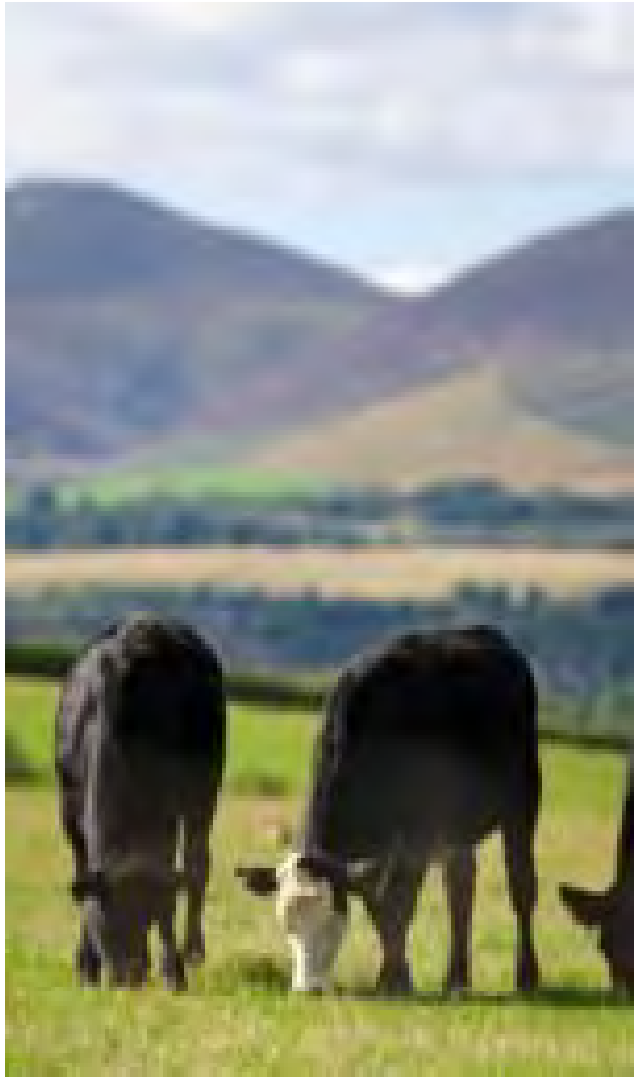
Work in progress - have sampled total of 178 beef cattle on 7 farms* by FEC and cAgELISA, have blood samples from 54

• Example farm:

FEC	cAg ELISA	Serum Ab ELISA	Number of animals
-ve	-ve	-ve	3
-ve	-ve	+ve	6
-ve	+ve	+ve	6

cAgELISA more sensitive than FEC in cattle?

* Rumen fluke present on ≥3 farms



Planned/future research?

- Pending BBSRC-IPA application with Liverpool, AHVLA and CEH (Diana Williams, PI), ”Improved control of liver fluke in cattle” – supported by EBLEX, QMS, HCC & AgriSearchNI
- Scottish Govt/RESAS Core Programme: On-farm fluke risk in Scotland (with JHI, SAC)
- Accept that current tests are imperfect and investigate/ evaluate alternatives? - DNA-based tests e.g. PCR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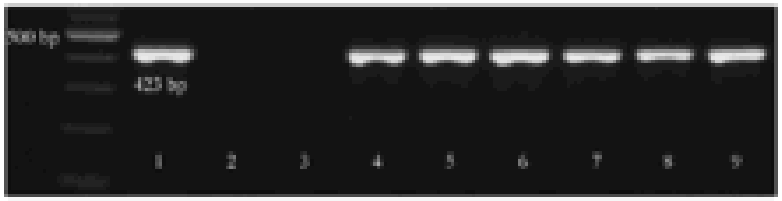
Veterinary Parasitology

Comparison of three different techniques to diagnose *Fasciola hepatica* infection in experimentally and naturally infected sheep

J.M. Martínez-Pérez, D. Robles-Pérez, F.A. Rojo-Vázquez, M. Martínez-Valladares

Authors claim “standard” PCR can detect *Fasciola* infection at 3wpi and “nested” PCR at 2wpi



Acknowledgements

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- Moredun staff & students
- Collaborators at SAC VIS, R(D)SVS, AHVLA & Liverpool Vet School

Thanks you for your attention!

Discussion Points from the SCOPS Liver Fluke Workshop 8-9th August 2102

1. Risk Factors

Farms and areas within farms posing a risk may be different this year given the weather patterns. Flooded areas may not be the high risk – the edges may be more of a problem as snails have been washed to the outer areas or they may even have been deposited in completely new places.

Poached areas are a significant threat. Changes in grazing patterns due to lack of aftermaths and higher stock numbers left on farms this autumn due to slow finishing rates.

The workshop discussed the need for a national forecasting system similar to the one that Ollerenshaw produced in the 1970's and 80's. Informing the model with data on fluke rather than just weather patterns is a challenge – huge resources involved in the Ollerenshaw predictions collecting fluke in the field.

Use of abattoir data a possibility (but would need to be traced to source rather than abattoir location).Dairy cows can be a good proxy for the distribution of liver fluke. Recent work at Liverpool taking incidence down to post code level suggests that the weather and infection patterns over a 5 year period are critical to accurate predictions, not just one season. Liverpool are now investigating farm specific factors to see if the accuracy of predictions can be improved further.

Gloworm project forecasting http://www.gloworm.eu/project

2. Diagnostics and Monitoring currently available

2.1 Acute Infections

No reliable diagnostics are available. Acute disease occurs very quickly (2 weeks) after the initial ingestion of large numbers of infective stages off pasture. Farmers in high risk areas need to be on high alert.

Post-mortem examination is the definitive diagnosis plus abattoir information on the state of livers in lambs and ewes in the late summer and early autumn.

Lambs are the most likely to be affected and also therefore the best sentinels.

2.2 Abattoir Feedback

There is a lot of potential to improve the value of feedback under the Food Chain Information initiative. For example currently sheep rejections due to liver fluke or

tenuicollis tend not to be differentiated and farmers still have to press for the information from some abattoirs.

Currently abattoir feedback is limited to a 'yes/no' liver fluke present or not. Delegates raised the question of whether in future this could be improved to give an indication of degree of damage / infection levels.

Collation of national information could give data on fluke distribution around the country if linked to EID and place of origin.

Feedback could also go direct to the farmers Vet – but there seems to be reluctance by farmers for that at this point in time – if we could overcome this then Vets would be better placed to advise their clients.

2.2 Faecal Egg Counts (Detection)

Normally this is only a *detection* of the presence of liver fluke eggs rather than a count of numbers. The value of counts and the cost /time involved requires further discussion / investigation.

Only 30% of faecal samples submitted to AHVLA are actually large enough to give optimal sensitivity. With 40g of faecal material sensitivity can be increased by 30-60%. Major action point to get this across to farmers and Vets. ***Bulk composite sample – 5g faeces from each of 10 animals = 50g in total***

Sedimentaion method is preferred - but there are a variety of methods used by different labs and there is a need for further investigation to get the best balance between sensitivity and cost.

False negative FECs are due to:

Samples are taken too early – no egg laying adult liver fluke

Cattle – liver fluke produce lower numbers of eggs and tend to shed intermittently

Dilute faeces – scouring animals

Dispersions in the herd – large individual variation

2.4 Clinical Chemistry (AST; GLDH and GGT)

These tools are only considered useful as supporting evidence because of the lack of specificity. May not show anything in chronic infections.

Some practitioners are using them to help monitor the situation on known fluke farms but it was felt this is only useful if there is a lot of prior knowledge regarding the history of liver fluke and other on-going surveillance (e.g. abattoir information).

AST/GLDH normally raised (3-4 times normal) from 13 days post-infection;

GGT takes longer (>40 days post-infection).

2.5 Serology

In England and Wales serology is only validated for use in cattle; in Scotland and NI serology is also used in sheep.

Can indicate exposure 2-4 weeks post-infection possibly lasting up to 6 months, but levels decline after 2-10 weeks so not always very reliable. Pooled or individual samples can be used.

2.6 Bulk Milk Tests (BMT)

An Elisa test is used for dairy cows and is also used for goats. It is an indicator of whether or not the herd has been ***exposed*** to Liver fluke infection. BMT should not be used alone as a determinant for the need to treat.

In NI it was reported that BMT was indeed leading some people to 'jump the gun', treating dairy cows unnecessarily.

3. Diagnostics and Monitoring – future tests

3.1 Copro-antigen Test

Detects fluke protein in the faeces and in theory should provide an earlier warning than egg detection because the latter relies on the presence of egg laying adults. Could potentially give more warning of fluke infection but may just be most useful as a cheaper / quicker means of establishing that a flock/herd has been exposed to liver fluke.

Claimed to give a positive test up to 4 weeks before Faecal Egg Detection. However, Moredun have been looking at this to validate in the field and as yet have not been able to repeat this advantage in terms of earlier warning. In NI the results have been a bit more encouraging – they report being able to pick up evidence of infection 3 weeks earlier in sheep, but still some way to go yet before it is fully validated.

One reason for the apparent lack of repeatability may be that infections are staggered in the field

One issue raised was that of individual samples v. composite samples. The former are more sensitive and give more reliable results but of course this adds to cost and complication.

3.2 Use of PCR

Several groups are looking to see if they can detect fluke DNA in faeces. Some reports of success but nothing beyond the research stage at present.

Fluke DNA in snails and/or on herbage also being investigated.

4. Triclabendazole (TCBZ) Resistance and Testing for Resistance

TCBZ resistance may well be regional, linked to frequent TCBZ use in high risk areas. This makes quarantine treatments of paramount importance when stock are moved.

Farms need to establish their TCBZ resistance status for liver fluke just as they do for roundworms. The tests available now are validated so this is possible – but for accuracy a Faecal Egg Count (rather than simple detection) is required and this is still quite expensive. More work is required on this aspect.

Currently there is no evidence of side resistance from TCBZ to albendazole.

Fluke population genetics are very different to roundworms – refugia based strategies are not applicable. Nor do we know how much of a risk under-dosing poses in the development of liver fluke AR.

Some people believe that there may be a relatively rapid switch from susceptible to resistant liver fluke within a population; others disagree and say it has been there for a long while but has been undetected – more research is required on the population genetics involved in liver fluke resistance.

Where a farm is shown to have TCBZ resistance it is vital that they look at the whole farm and clearly identify the high risk areas and take steps to eliminate these areas where possible (fencing, drainage etc.) to reduce reliance on chemical treatments.

5. Testing for Resistance

Sheep are the best sentinels to use in AR investigations.

FECRT test done 21 days after treatment – Liverpool University have validated a composite ***count*** FECRT. <80% reduction is the normal threshold for AR detection in liver fluke and this then requires further investigation, not knee jerk reaction and assumption of AR.

One important query was raised over the viability of Liver fluke eggs post-treatment. We do not know if the eggs seen in the post treatment samples of an FECRT are actually viable or if the treatment rendered them unviable – this would make a huge difference to interpretation of results.

We need validated AR tests for closantel and nitroxynil – AR to these has been reported in other parts of the world so this must be considered.

Histology

Fairweather and Hanna work in NI. Differences in the reproductive system seen under the microscope is the indicator in the test. Current work is on mature fluke but the aim is to look at younger specimens. Can only be used in experienced hands, not applicable for general use at this stage.

Molecular TCBZ resistance markers are being sought by research groups.

6. Management Options

The group were focussed on the need for farmers to use management options wherever possible as the first line of defence against liver fluke.

Fencing areas off permanently is not so straight forward these days with SFP monies at stake – some dialogue with the RPA may be necessary on this point.

Minimising future impact – reducing eggs shed next spring using an adulticide

Summer treatments to reduce egg shedding?

7. Products and Product Choices

Greater clarity on which products do what is required. There is much confusion with different withdrawal periods for the various products containing TCBZ and also the age of flukes killed (e.g. TCBZ pour-on only down to 7 week old flukes. Farmers need clear information as given by the Eblex and SCOPS leaflets.

Concern was expressed by the group about the efficacy of pour-on treatments in cattle and the risk of over-dosing with closantel. Several instances were known to the group (blindness) and the need for SARS reports to VMD when this occurs was underlined.

In sheep, over dosing with closantel will cause deaths so splitting groups where there is a wide weight range is very important.

The group recommended that a colour or symbol coding system was considered (similar to the roundworm groups) to help farmers and their Vets/advisers make the right product choices.

Products available for dairy cows are very limited (albendazole) and with new MRL (maximum residue levels) levels on the horizon this situation could get worse in the near future – this would include calves destined for dairy production.

Use of (fluke and worm) combinations to be discouraged to reduce the inadvertent selection of roundworms for resistance and improve the timing of treatments. The possible exception

to this is the use of BZ/closantel combination because of the synergistic effect of the combination – may be particularly helpful on TCBZ resistant farms.

8. **Quarantine**

- General recommendation is to use TCBZ + an FECRT to check it has worked properly
- Use of two actives also a possibility (TCBZ + another) but these should be given a few days apart as there is no indication that it is safe to give them on the same day 'sequentially'
- Two doses of closantel 6 weeks apart with intervening time on dry (low risk) pastures.
- Housing option for cattle and then could delay treatment if not at high risk in terms of levels of infection (closantel; nitroxynil or clorsulon are treatment options).

9. **Rumen Fluke (paramphistomum)**

Important to keep this in perspective – there is a danger that it's profile is over exaggerated, but it is becoming more prevalent.

There is a risk of misdiagnosis because the rumen fluke eggs are similar to liver fluke.

They may be more of an issue in recently flooded land (the intermediate host is a snail that likes deep water) which has important indications for the conditions in 2012 with widespread flooding in some areas of the country.

Losses are due to damage to the duodenum from very heavy infestations; 20% mortality in cattle reported by SAC in one outbreak.

The SCOPS group would like to sincerely thank all those who gave up their time at short notice to attend this workshop.

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Roger Daniel, AHVLA

John Fitzgerald, SCOPS, RUMA and Workshop Chairman.

Harriet Fuller, SCOPS and Practitioner

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Andrew Robinson, Practitioner

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Mike Taylor, SCOPS and Parasitologist

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