resistance
frequency
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isolate
Nematodirus
scouring
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Resistant
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Benzimidazoles
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species
gastrointestinal
risk
overwinter
UK
faecal
BZ-resistance
battus
polymorphisms
prevalence
spring
egg
identification
populations
farms
refractory
SNP
BZ-s
arctic
in-depth
species
dissemination
development
results
young
spontaneous
management
lambs
survey
multiple
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PCR
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environmental
technologies
failure
livestock
selection
rapid
alleles
resistant
samples
DNA
disease
eggs
treatment
genotyping

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Lynsey Melville

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Nematodirus battus

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- Typically infects young lambs, 6-12 weeks old
- Symptoms
  - Acute scouring
  - Dehydration
  - High mortality rate
- Commonly controlled using benzimidazole (1-BZ)

N. battus life cycle

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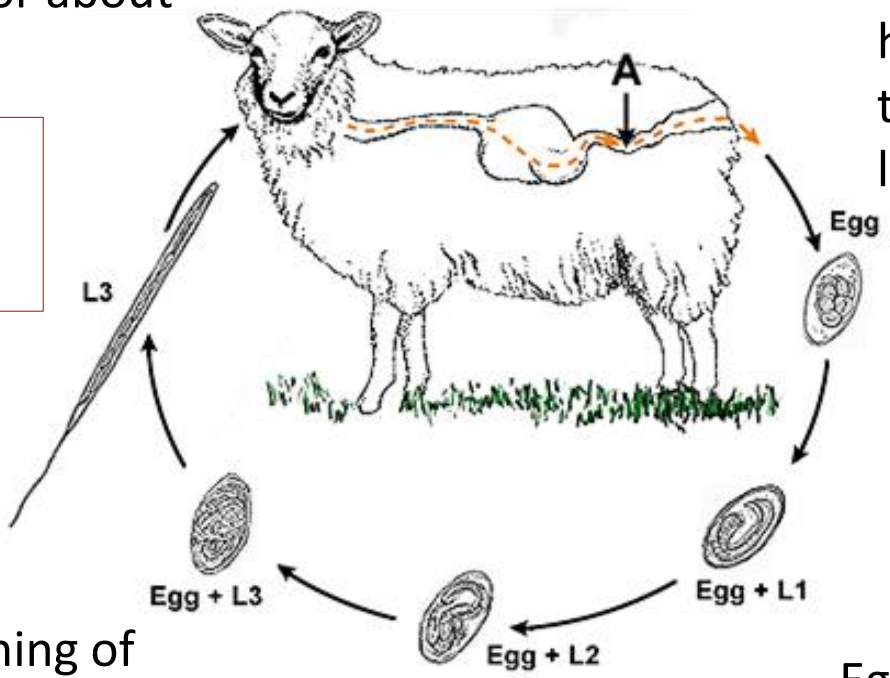
High numbers of larvae on pasture – persist for about 1 month

Infectious larvae on pasture in autumn/early winter



Acute disease occurs when synchronous hatching coincides with the grazing of young lambs

Low level infections in older animals

Synchronous hatching of developed eggs occurs **April-June**
(Hatching = chill followed by a mean temperature >10^{o}C)

Hatching out with the expected conditions/ time points

Eggs typically over-winter on pasture and **hatch next spring**.

Nematodirus research

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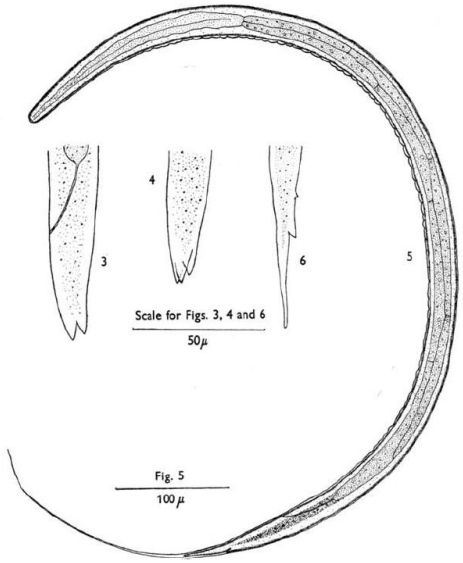
- Research conducted in 1950's

Journal of Helminthology, Vol. XXVIII, *Nos.* 3/4, 1954, *pp.* 119—122.

A Further Description of *Nematodirus battus* Crofton and Thomas, 1951

By H. D. Crofton and R. J. Thomas
Department of Zoology, University of Bristol



- Research papers published in the last 20 years:
  - *Nematodirus battus* – 21
  - *Teladorsagia circumcincta* – 597

Changing behaviour

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Previously:

Spring-time disease of young lambs

Now:

Still a spring-time disease BUT...

- Infection of older animals throughout the year
- Autumn *Nematodirosis*
- Emergence of BZ-resistant *Nematodirus*

Research is required to re-characterise this parasite and to understand what is driving these changes

Project aims

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1. Create an accurate picture of the prevalence of BZ resistance in UK *N. battus* populations

2. Identify potential risk factors relating to the development and spread of BZ resistance in *N. battus*

3. Develop and evaluate a high-throughput diagnostic molecular assay for the assessment of the BZ resistance genotype of *N. battus* populations

Benzimidazole resistance

Benzimidazole

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- Mode of action
  - Inhibition of microtubule polymerisation
  - Colchicine binding sites on β-tubulin

- Benzimidazole resistance:
  - Single nucleotide polymorphisms (SNPs) within the β-tubulin isotype 1 gene
  - Alter the conformation of the binding sites

F167Y, E198A & <u>F200Y</u>

Benzimidazole resistance

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- Majority of farms in the UK have BZ-resistant GIN

- Resistance development:
  - Environmental
  - treatment
  - management factors

BZ-resistance in *N. battus*

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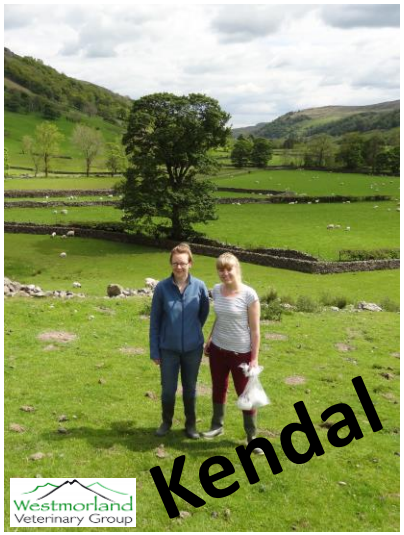
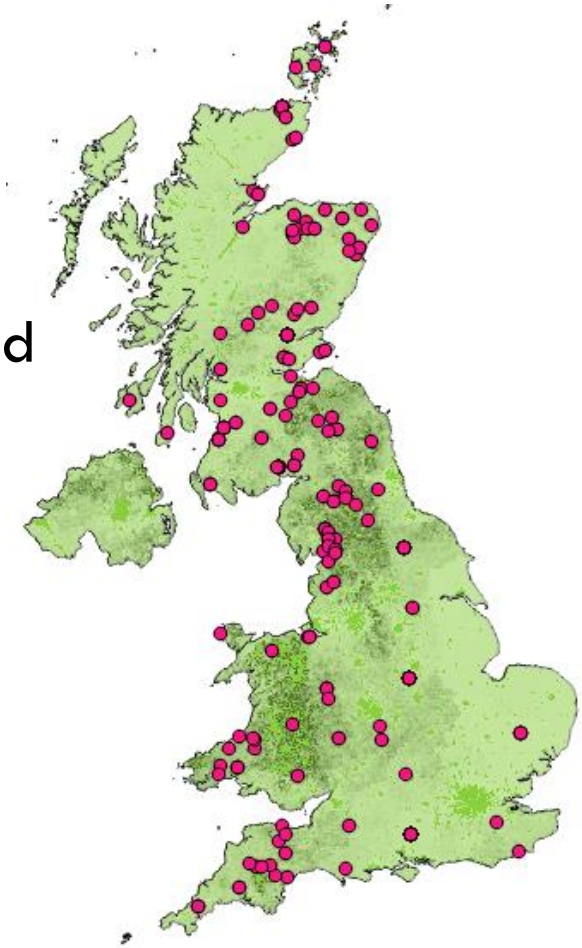
- Previously believed refractory to the development of resistance
- First case of resistance identified in 2010
- BZ-resistance was confirmed by dose and slaughter test at Moredun

Sample collection

- Veterinary Investigation centres
- Regional advisors
- Sampling trips
- Animal and Horticultural Development Board





297 *N. battus* populations from 284 farms

Sample processing

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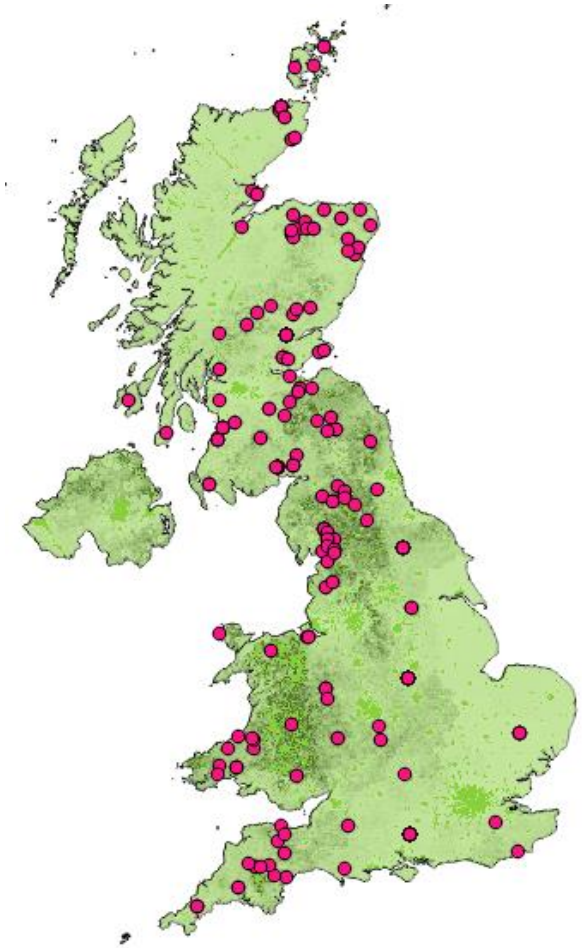
- Pyrosequencing used to analyse the F200Y SNP in N. battus (Morrison et al. 2014)
- 30 individual eggs/L3 analysed per population



297 *N. battus* populations from 284 farms

Prevalence of BZ-r alleles

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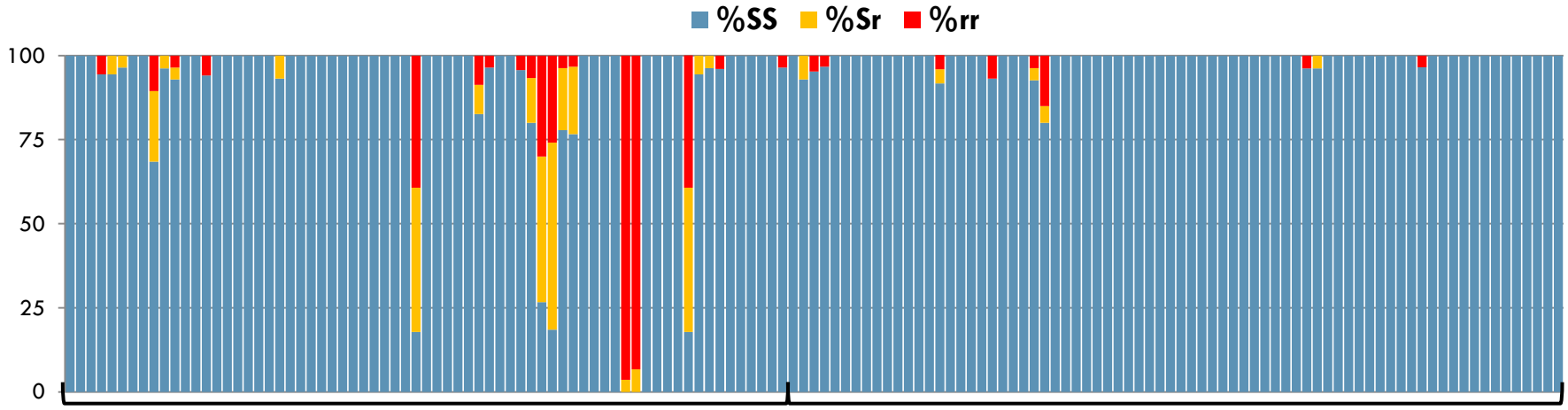
- Low resistant allele frequency ~3% UK overall
- Focal regions of high frequency
- Resistant allele identified in ¼ of populations tested



England & Wales

Mean r gene frequency 5%
range 0-98%

Scotland

Mean r gene frequency 1%
range 0-18%

Prevalence of BZ-r alleles

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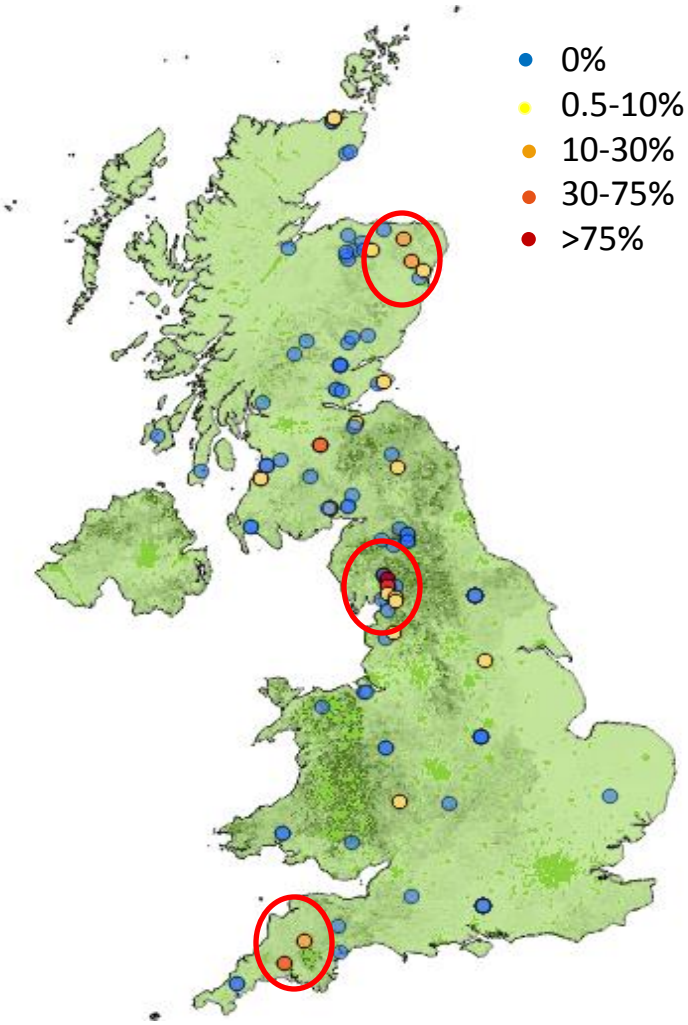
- BZ-r alleles identified at low levels across the UK
- Several potential 'focal regions' of resistance have been identified
- Origin of resistance unclear
  - Multiple spontaneous, recurring mutations
  - Pre-existing mutation
  - Dissemination from a single source



Prevalence of BZ-r alleles

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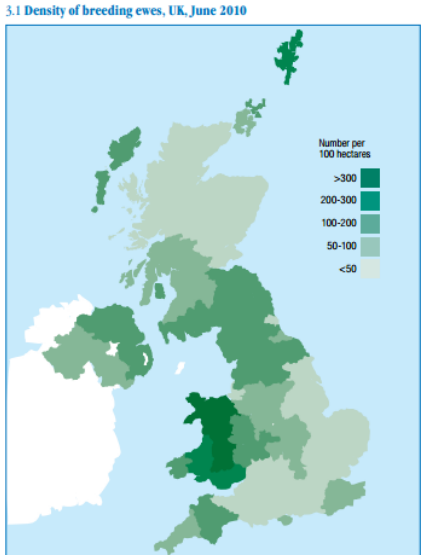
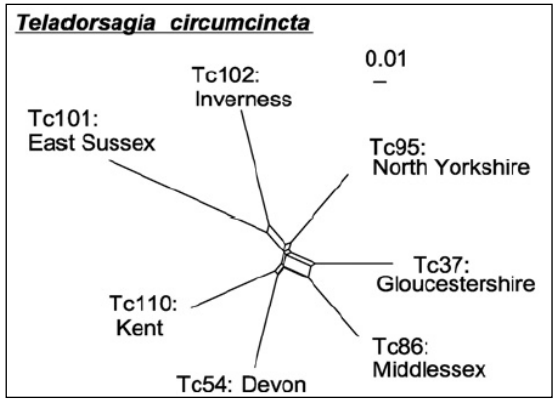
Next steps…

- Further sampling to ensure accurate representation of UK *N. battus* populations



AHDB yearbook 2014: sheep

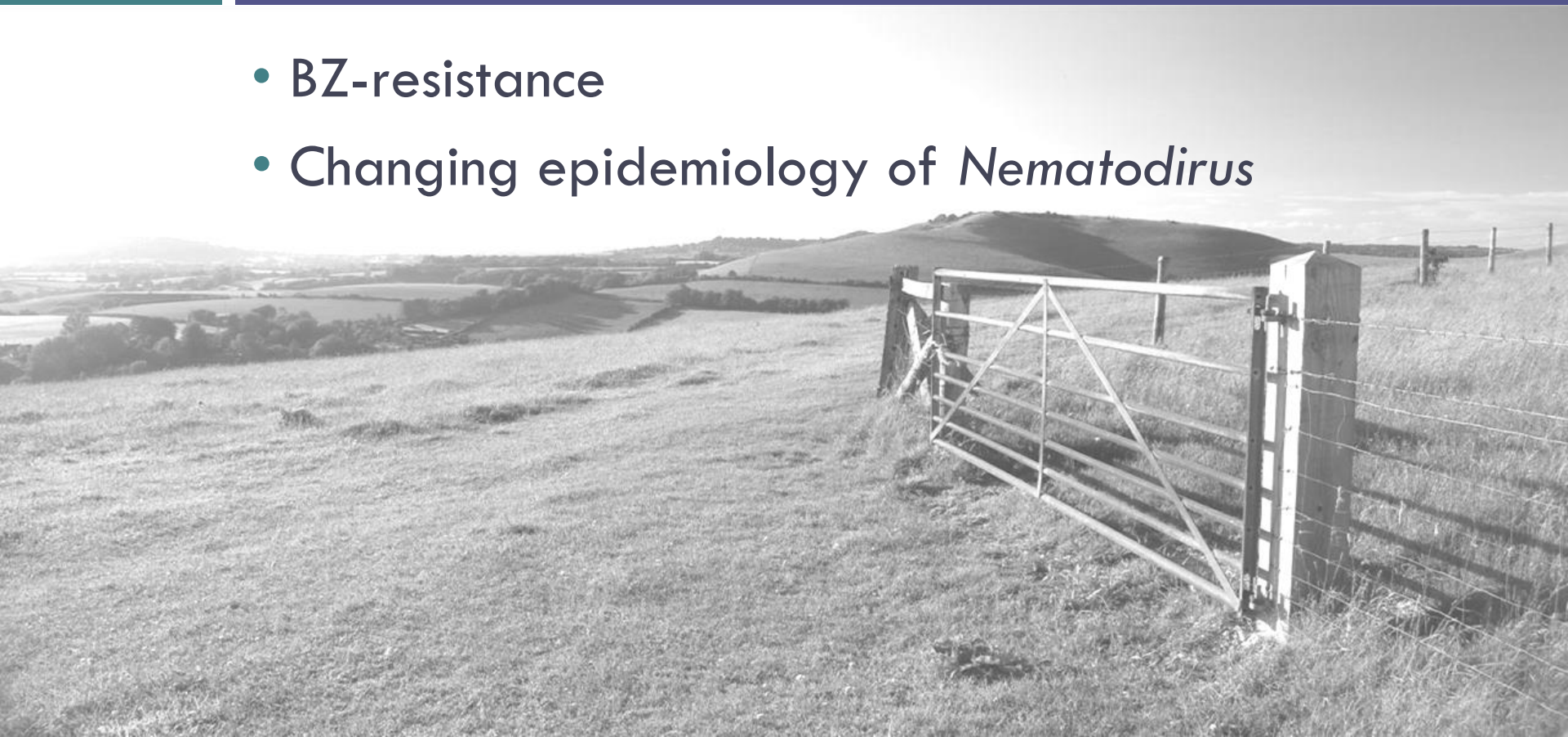
- Collaboration with University of Calgary to conduct sequencing of *N. battus* populations to investigate population structure & potential origins of resistance



Redman et al. 2015

Risk factors

- BZ-resistance
- Changing epidemiology of *Nematodirus*



Risk factor analysis

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Analysis of hatching patterns

BZ-r
Nematodirus

Hatching patterns

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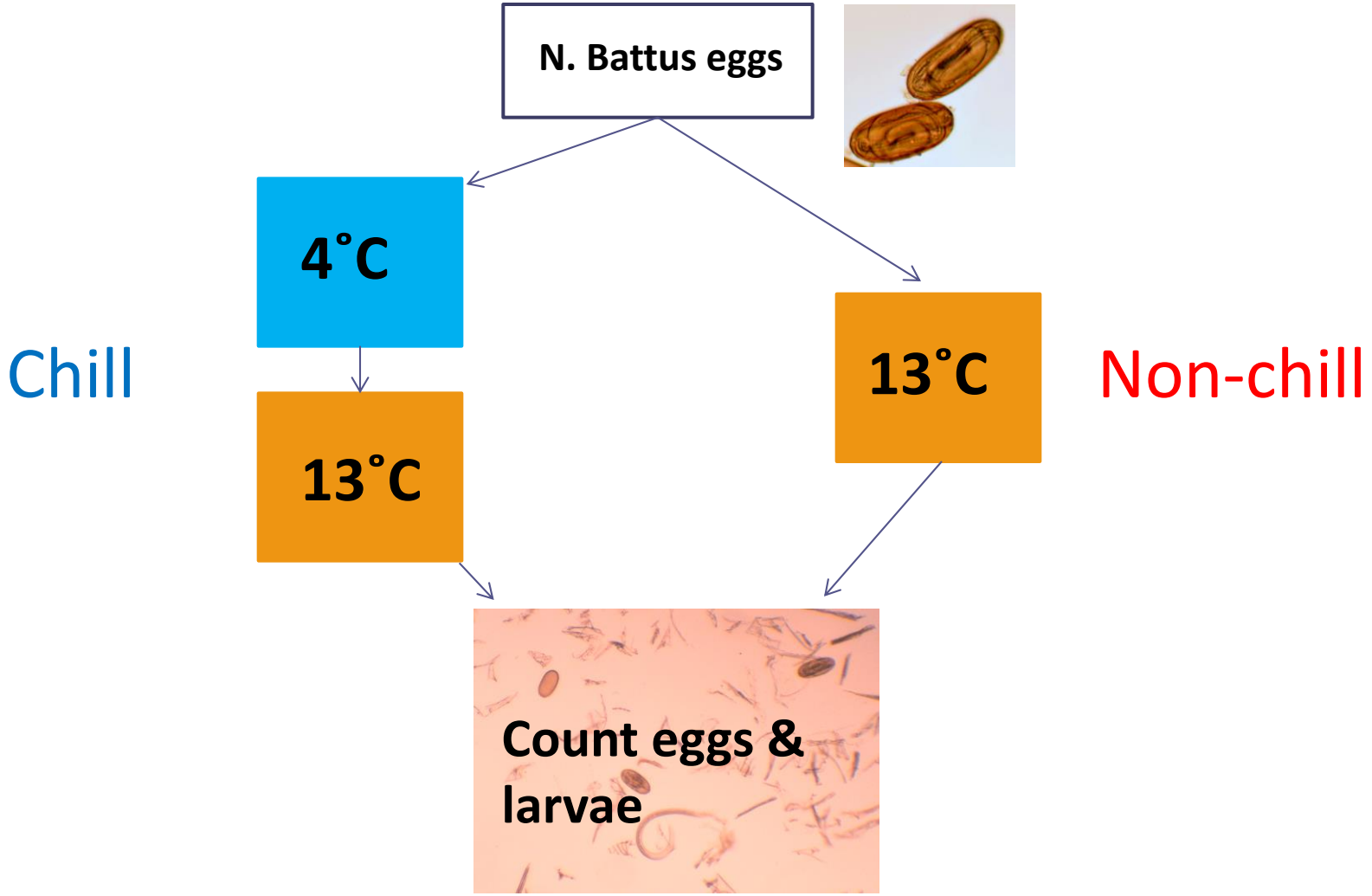
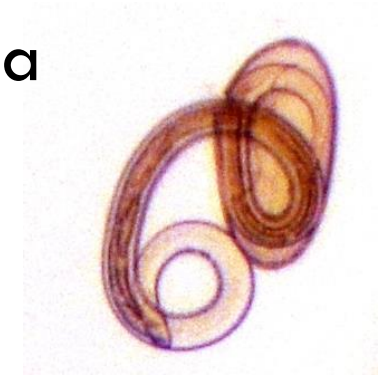
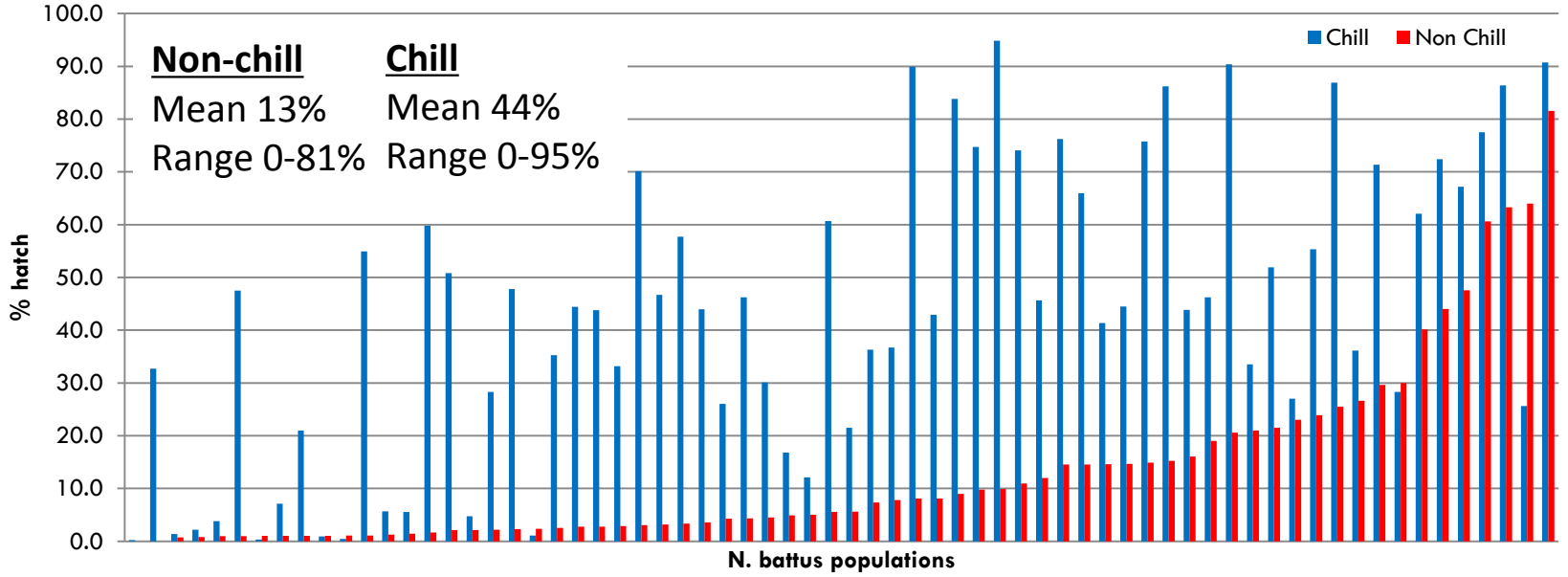


Hatching patterns

- Historically - 10 days at 10°C following a period of chilling
- A significant proportion of eggs were able to hatch without a chill stimulus





Hatching patterns

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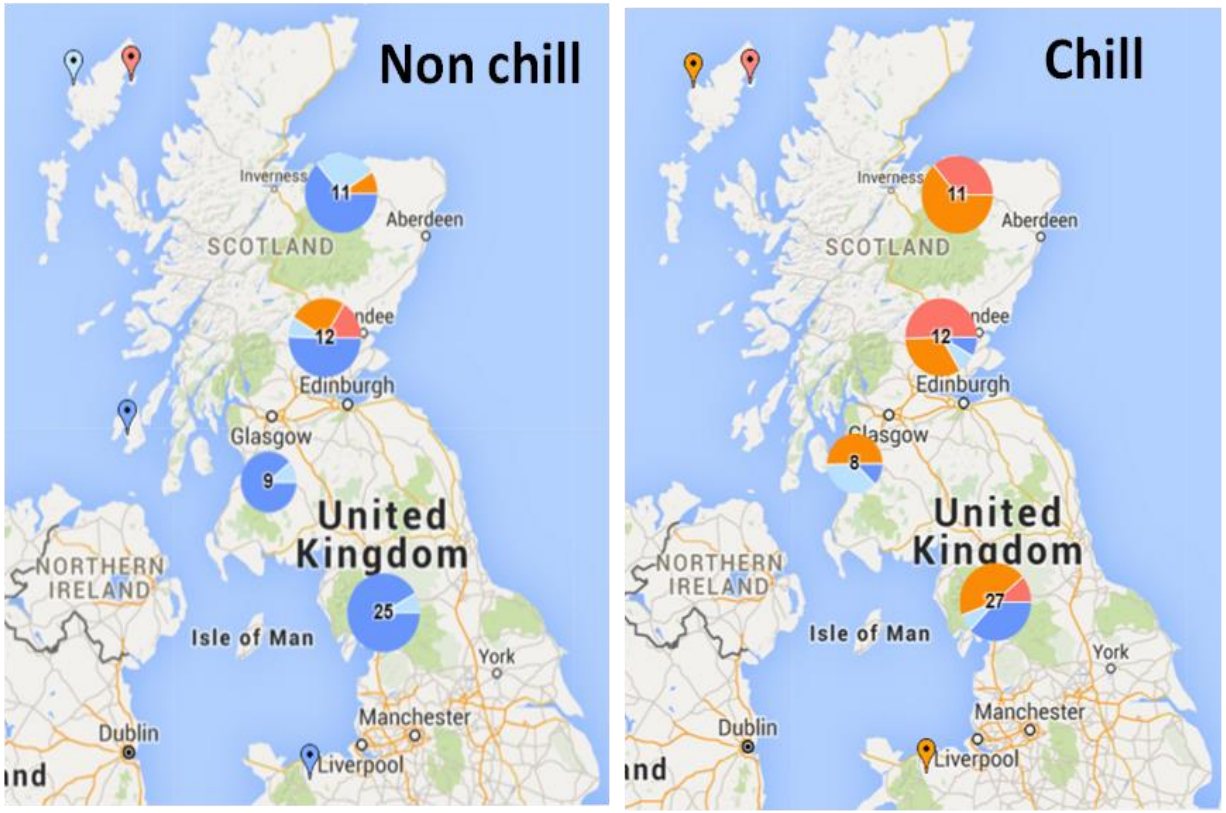

Proportion of eggs hatched:

0-15% 15-30% 30-60% >60%

Hatching vs. genotype

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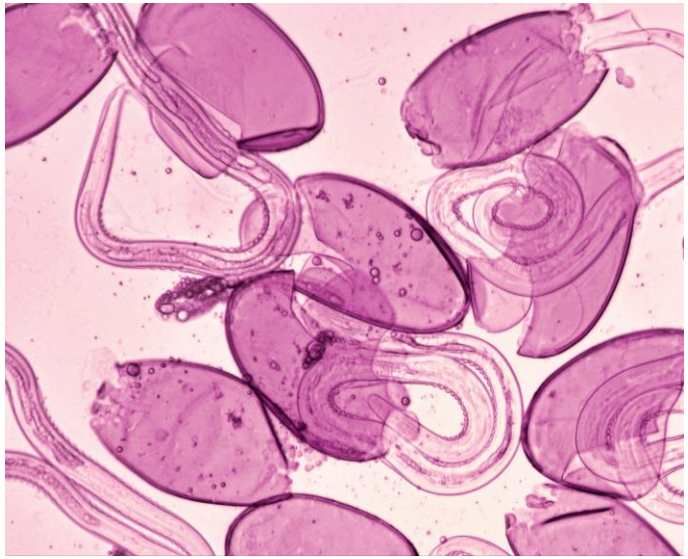
- Is the emergence of resistance a result of the recent change in hatching patterns of *N. battus*?

- Increased number of generations possible per annum
- Increased selection pressure



- No correlation between resistant allele frequency and proportional hatch under non-chill conditions

Hatching vs. genotype

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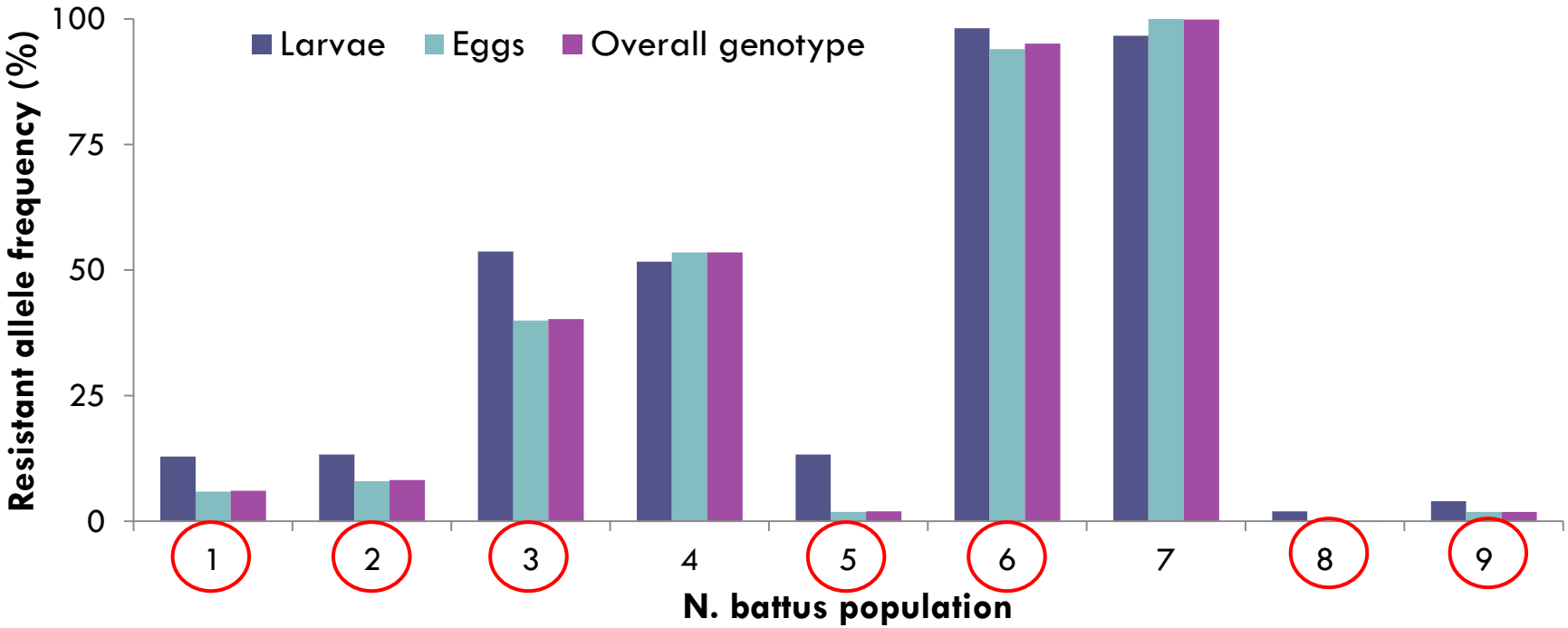
- Resistant allele frequency higher in larvae hatched under non-chill conditions than unhatched eggs



- Wilcoxon signed rank p=0.044

Autumn hatching

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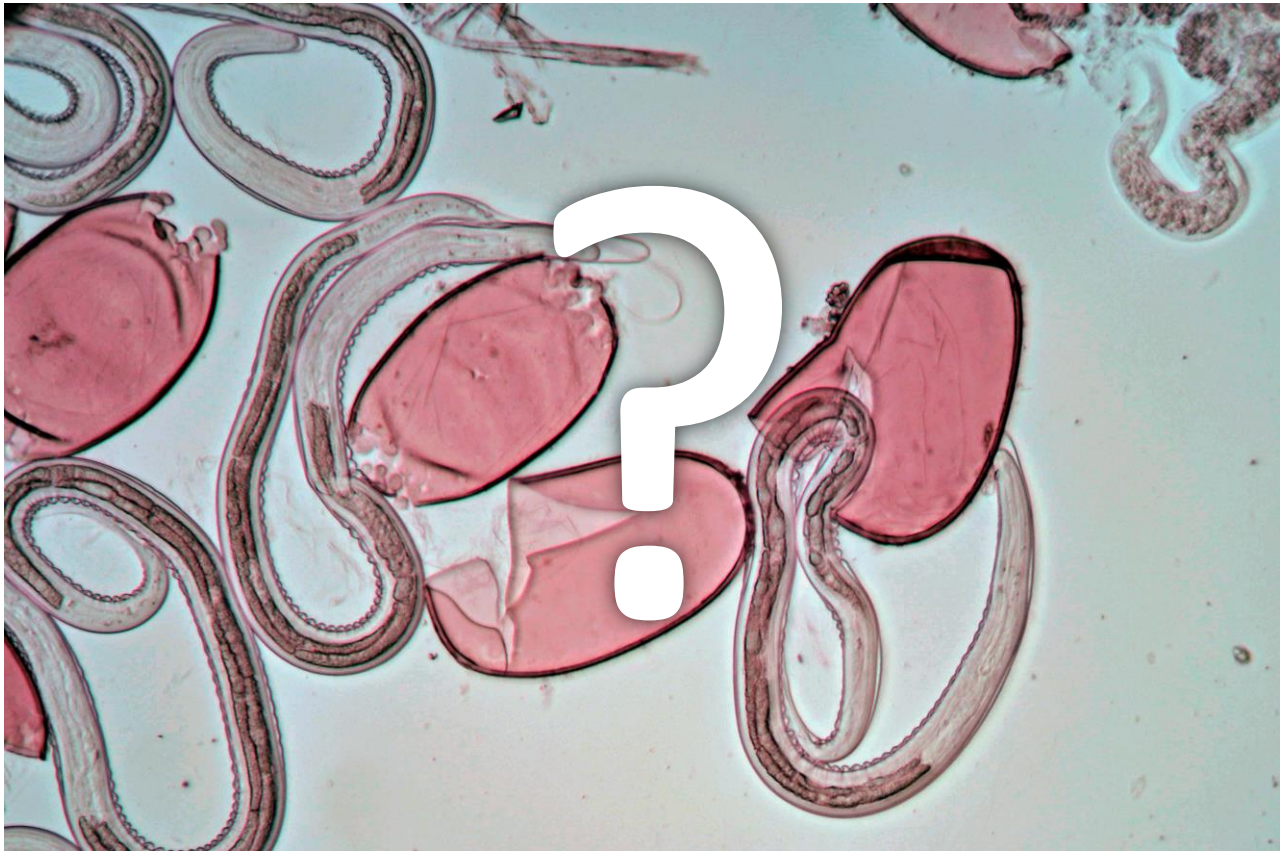

Autumn hatching

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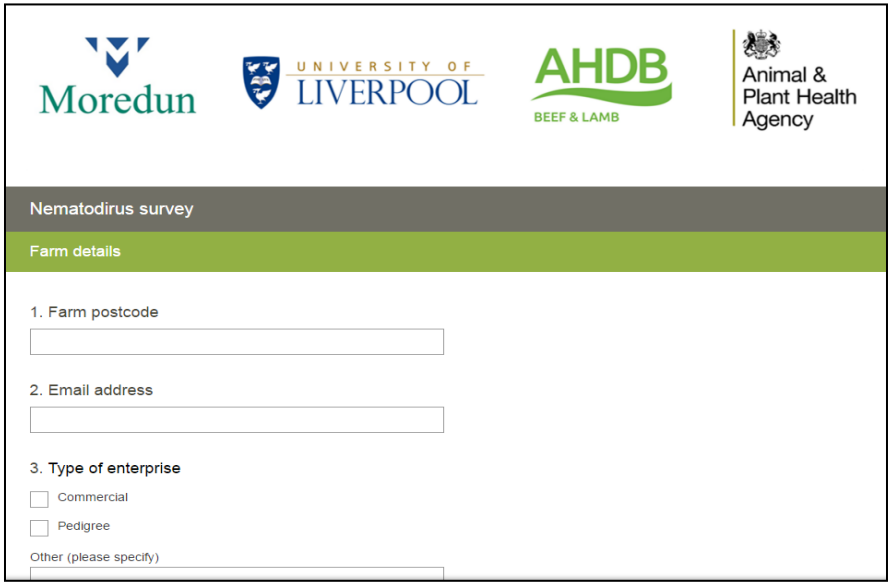
Parasite population structure

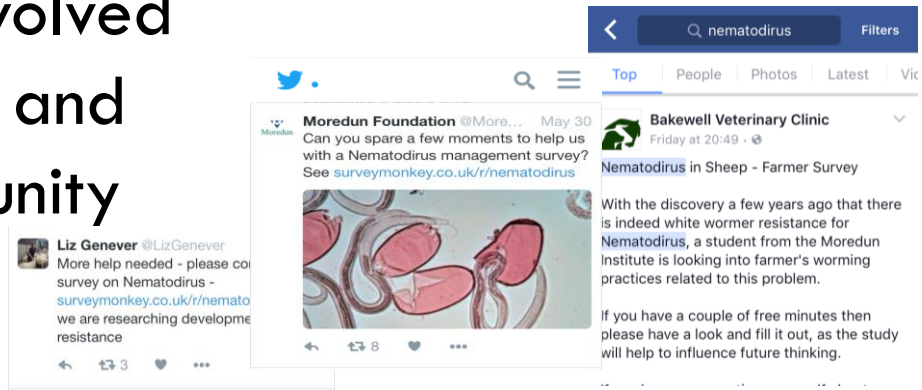
Questionnaire

Questionnaire

- Online survey
  - Grazing management
  - Worm control practices
  - Farm demographics



- Distributed to farmers involved in our genotyping survey and the wider farming community



Outcomes so far...

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<u>Prevalence survey</u>

- ✓ BZ-r alleles at low frequency with focal regions of higher frequency

<u>Risk factor analysis</u>

- ✓ Variation between populations in ability to hatch without a chill stimulus
- ✓ Emergence of resistance not thought to be linked to changes in hatching patterns
- ✓ Resistant allele not believed to carry a fitness cost
- ✓ Questionnaire currently live

End goal…

- Current prevalence of BZ-resistance in *N. battus*
- Evaluate risk factors
- Novel diagnostic

➢ Aim – to inform future best practice advice & reduce the economic impact of BZ-resistance in this species

Acknowledgments

Moredun

Supervisors:

Dave Bartley

Jan van Dijk

Sian Mitchell

Funders:

AHDB

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Thanks to:

Moredun Parasitology team

Farmers involved in this project

UNIVERSITY OF LIVERPOOL

Animal & Plant Health Agency