Detection and further molecular characterisation of anthelmintic resistance *in Nematodirus battus*

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Nematodirus battus

Nematodirus battus is an important nematode parasite found in the small intestine of lambs. It can cause diarrhoea, ill thrift and death. It has an unusual epidemiology; historically eggs have required chilling followed by a period of warmer temperatures in order to hatch to release their infective larval stage.

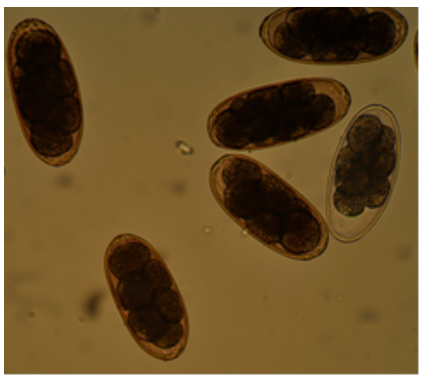

Fig 1. *N. battus* eggs from faeces.

Fig 2. Lambs 6-12 weeks old are typically affected.

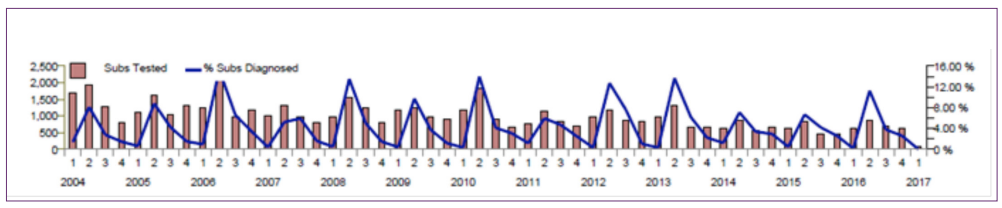

Fig 3. Incidents of PGE-nematodirosis in GB as diagnosed by APHA and Scotland's Rural College (SRUC), as a percentage of diagnosable submissions, 2004 to date.

Anthelmintic resistance

Benzimidazoles, (1-BZ) remain the anthelmintic of choice for the control of *N.battus* due to it's high safety index and high efficacy on most farms. This is despite resistance to benzimidazoles in other gastro-intestinal nematodes in sheep in the UK.

The first instance of anthelmintic resistance in this parasite, in the world, was detected by the diagnostic scanning surveillance service of the APHA and confirmed by collaboration with the Moredun Research Institute.

Further work has elucidated the molecular basis of this resistance to the benzimidazole class of anthelmintics and allowed investigations into its prevalence on a large number of populations (n=339) from across GB. 1-BZ-resistance is conferred by a mutation within the β-tubulin isotype 1 gene and a genotyping assay has been developed to assess the mutation frequency from individual eggs and /or larvae of *N. battus*. Resistant genes have been detected at a low prevalence (~5%) but on around a third of the farms examined with several potential 'focal regions' of high level resistance identified.

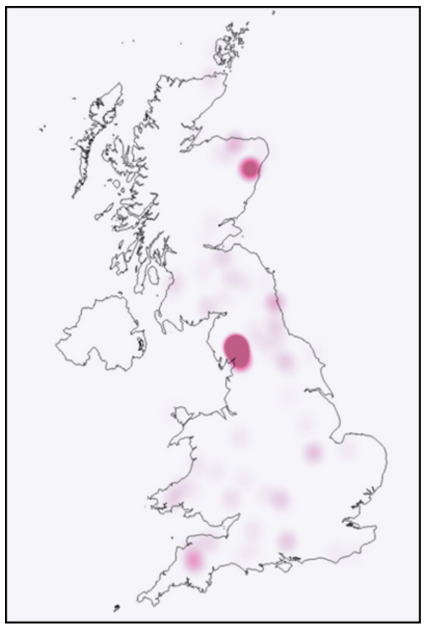

Fig 4. 'Heat' map of resistant allele frequency from populations of *N. battus* analysed.

Currently we cannot correlate gene frequency with clinical efficacy of BZ anthelmintics. It is also unclear as to how the anthelmintic resistance has arisen in muliple areas after almost 60 years of usage against this parasite. There are a number of possible theories:

- Multiple spontaneous, recurring mutations
- Dissemination from a single source
- Selection on pre-existing mutation

Hatching behaviour

Nematodiriosis is typically described as a spring-time disease of young lambs transmitted from one year's lamb crop to the next. However, in recent years the behaviour appears to be changing and is now regularly observed out with spring. The hatching of *Nematodirus* eggs was historically believed to, and may still, be highly dependent upon climatic conditions but the pattern of hatching is changing.

Populations were examined for their ability to hatch with/without the need for a chilling stimulus. *N. battus* eggs from 90 populations were collected from across Scotland and England.

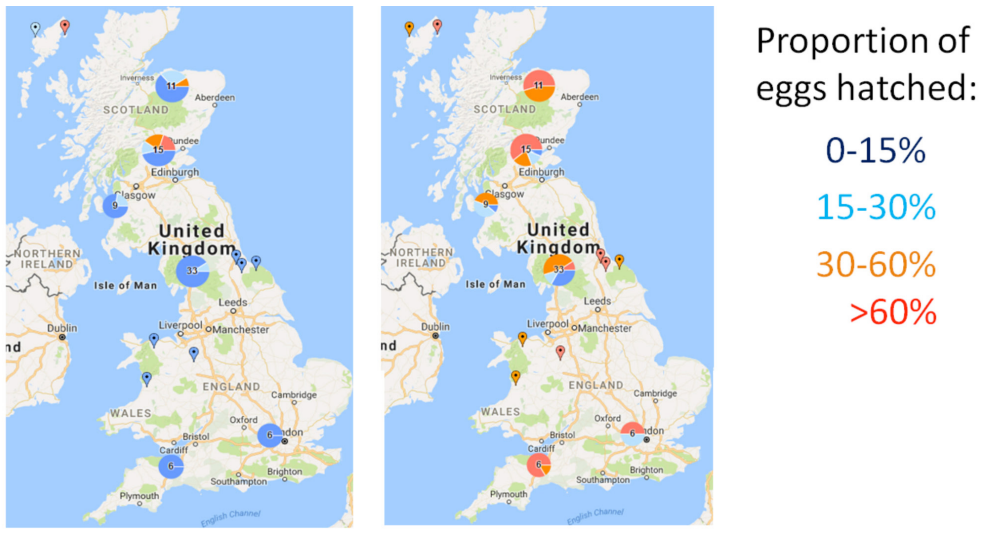

Fig 5. Proportion of eggs hatched with and without a chill stimulus by farm location. Pie charts represent hatching proportions from a number of farms within the area.

Conclusions

- Resistant alleles in *N. battus* are currently at a low prevalence throughout the UK but with isolated populations containing higher frequencies.
- Further work is ongoing to try to elucidate the origin of the mutations and possible farm-level risk factors associated with BZ resistance in this parasite.
- This is the first time molecular tools have been available at an early stage of resistance detection.
- Changing pattern in egg hatching behaviour, away from historical perception, is occuring and may explain clinical signs being observed in animals in the autumn/winter.
- Further analysis is required to characterise any link between resistant genotypes and hatching behaviour.

References

Benzimidazole resistance in *Nematodirus battus*. Mitchell, S., Mearns, R., Richards, I., Donnan, A.A., Bartley, D.J. Veterinary Record (2011) 168 (23) 623; DOI: 10.1136/vr.d3584

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