New tools for sustainable control of liver fluke in ruminants

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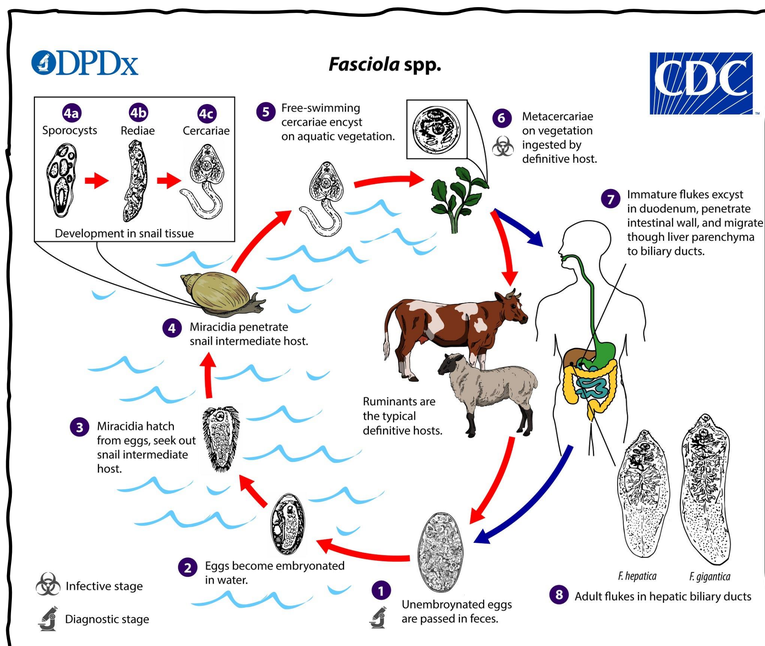
Research Questions

What is the existing population structure of *Fasciola hepatica*, and how does this impact parasite gene flow in cattle and sheep in different geographical areas in the UK? How can we improve the existing "fluke forecasts" models using climate and genetic data?



Food security is a significant challenge

- Agri-food system contributes £115bn, which is 6.0% of the UK economy
- Livestock part £14.7 billion in the UK economy
- Expansion in the global population
- Parasites compromise animal health
- Zoonotic importance

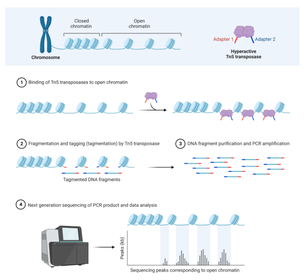
Economic losses by Liver fluke in the UK

- Liver fluke is an endemic problem in ruminants in the UK
  - Compromising production
  - Costing the cattle industry £13 to £40 million annually
  - Reducing net profits of dairy farms (12%) and beef farms (6%) (Shrestha et al., 2020)
  - Condemnation of infected liver by 30% (Skuce and Zadoks., 2013

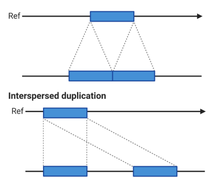
Needs addressed by the project

- Limited data available for parasite genetic diversity and gene flow
- User-friendly genetic markers with good genome coverage required for in-depth analysis of *F. hepatica* population structure
- Population genetics and multiplicity of infection will provide insights into infection rates
- Need to update existing "fluke forecasts" based on models using climate data

Aim 2 and 3: Whole Genome sequencing, development, and validation of molecular markers; and to understand population genetics, multiplicity of infection, and genetic flow of *F. hepatica* in the UK



Whole genome sequence from *Fasciola* worm tissue DNA



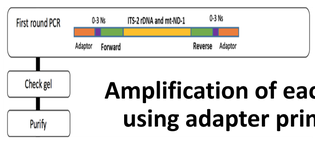
Genome mapping with reference genome from database https://parasite.wormbase.org

Identification of fragments containing SNPs from aligned genome

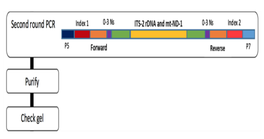
Primers designing from evenly spaced intervals, multiple regions of genome

Multiplexing and optimization of designed primers

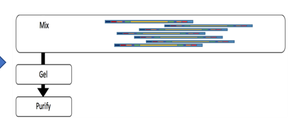
Application of mt-ND-1, ITS2, and optimized markers on filed isolates



Amplification of each loci using adapter primers

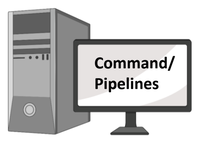


Amplicons generated from each sample with barcoded PCR



Pooling of all samples

Sequencing on the Illumina MiSeq Platform



Bioinformatics Analyses

Few parts of the figure taken from (Rehman et al., 2020)

Aim 1: Development of rapid diagnostic tools for the identification of *F. hepatica* from the faecal samples

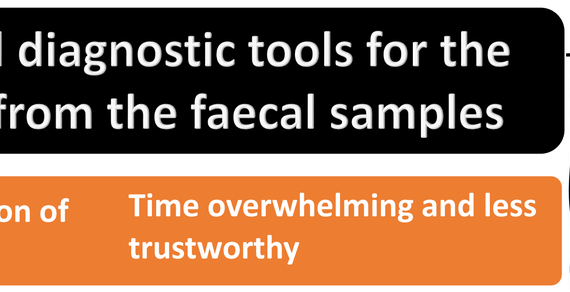
Identification Methods

Morphological identification of *Fasciola* eggs	Time overwhelming and less trustworthy
Development of qPCR and LAMP assay	More sensitive and specific for initial screening

Development of qPCR and LAMP assay using mitochondrial mt-ND-1 primers to rapidly diagnose *F. hepatica* from faecal samples.

Sensitivity of qPCR assay

Sensitivity of LAMP assay



qPCR showed sensitivity up to 19.6 femtograms of *F. hepatica* DNA

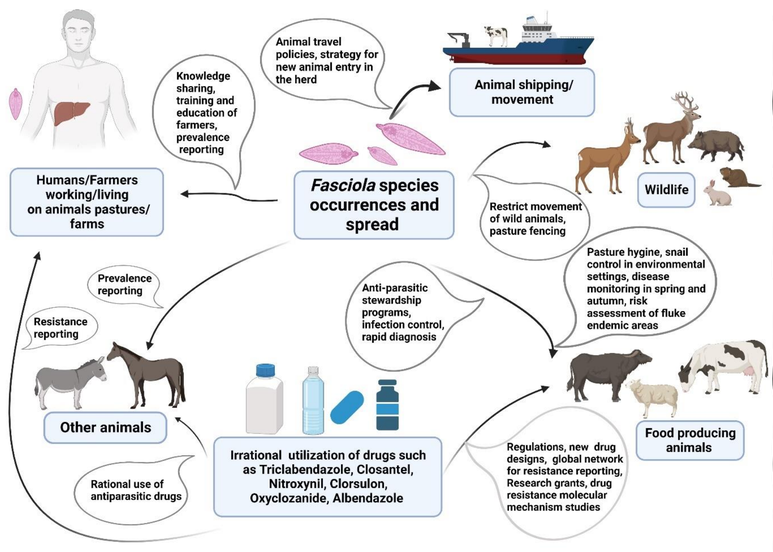
LAMP assay showed sensitivity up to 12 picograms of *F. hepatica* DNA

Work under progress

- Specificity analysis of qPCR, and LAMP assay with other flukes and nematode species
  - *Calicophoron daubneyi*, *Paramphistomum*, *Explanatum explanatum*, *Dicrocoelium dendriticum*, *H. contortus*, *Teladorsagia circumcincta*, *Trichuris*, *Ascaris*
- Processing of field samples for qPCR and LAMP assay; and DNA extraction as follows:
  - DNA isolation from faecal sedimentation material via the direct lysis method
  - DNA isolation from faecal sedimentation material via DNeasy PowerSoil Pro Kit
  - DNA isolation directly from faecal material via DNeasy PowerSoil Pro Kit

Aim 4: To integrate genetic data into climate-based models of *F. hepatica* transmission to improve "fluke forecasting" and control measures

- Commonly used Ollerenshaw model in the UK
  - Mt = N(R – PE + 5)
    (Ollerenshaw and Rowlands, 1959)
- In the UK, the climate is changing, with shorter and warmer winters, and a rise in rainfall in the spring, winter, and autumn resulting in a 4-week expansion of the pasturage growth (Kenyon et al., 2009)
- Fox et al., (2011) describe that most of the UK was free from fasciolosis in the 1970s,
- In 2000, only occasional losses
- Climate-driven forecasts predicted severe epidemics in Wales by 2050



Control measures and interventions strategies to restrict the spread of *Fasciola*

References

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