FLUKE WARS

EPISODE 2: ATTACK OF THE CLONES

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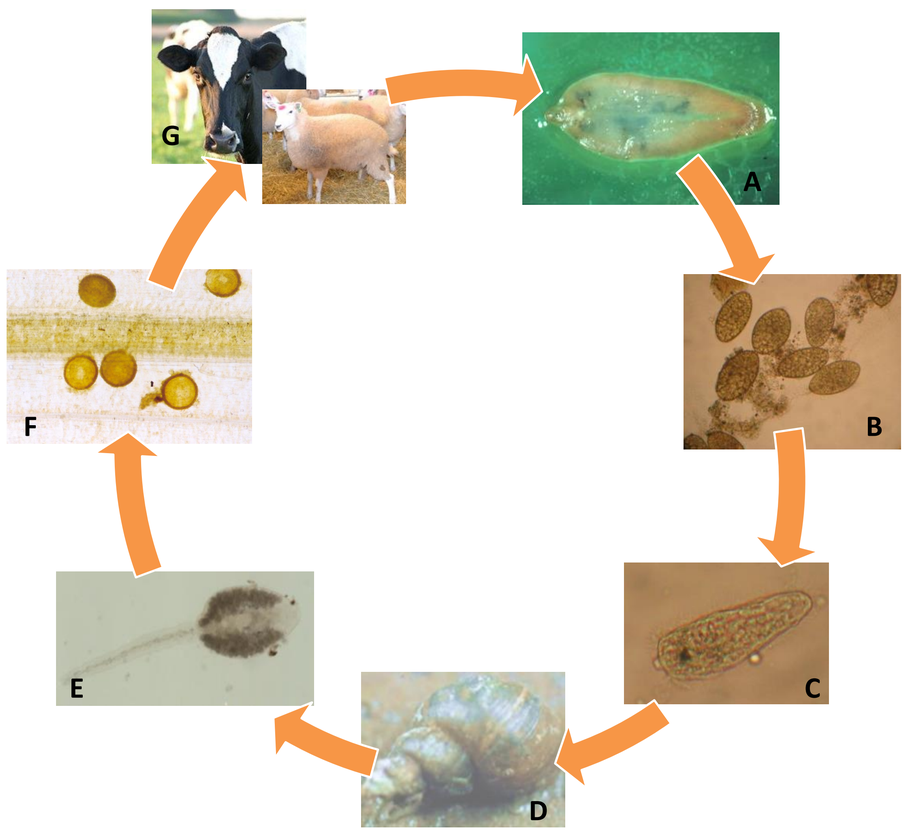
Fasciola hepatica (liver fluke) is a parasite that, in the UK, predominantly infects the livers of sheep and cattle. An essential stage of the liver fluke life cycle, where clonal expansion of the parasite occurs, takes place within the snail *Galba truncatula*. This has the potential to affect the genetic variation we see. In this study we genotyped 1579 parasites using a panel of eight genetic markers. Whilst we found a significant amount of genetic variation, we also found 96 sets of clones, which equated to 251 clonal parasites. This most likely occurs as a result of clonal expansion within the snail.

Introduction

In the UK *Fasciola hepatica* (liver fluke) causes an important parasitic disease of sheep and cattle. It costs the UK farming industry approximately £200 million per annum, and is a welfare issue to the animals it infects.

These parasites have developed resistance to the drugs that we use to eliminate them. As this resistance trait is carried within the parasites' genes, it is important to understand how genes flow and how the **liver fluke life cycle** affects the genetics of the parasite population.



Adult fluke (**A**) live in the livers of sheep and cattle (known as the definitive host). They produce eggs (**B**) which are passed onto pasture in the faeces of the definitive host. Some development occurs and a miracidia (**C**) hatches from the egg. The miracidia seeks out the snail intermediate host (**D**). Development occurs in the snail and multiple cercariae (**E**) exit the snail, these cercariae are all clones of the miracidia that entered the snail: we call this clonal expansion. The cercariae encyst on grass as metacercariae (**F**) which is the infective stage and is eaten by the definitive host (**G**) as they graze.

Aims and Objectives

Whilst we know that clonal expansion occurs within the snail there is little information as to how this impacts genetic diversity, for example do we find parasites with the same genotype in the definitive host in the UK? Therefore we sought to genotype adult *F. hepatica* collected from sheep and cattle and determine how many of the parasites had the same genotype.

Materials and Methods

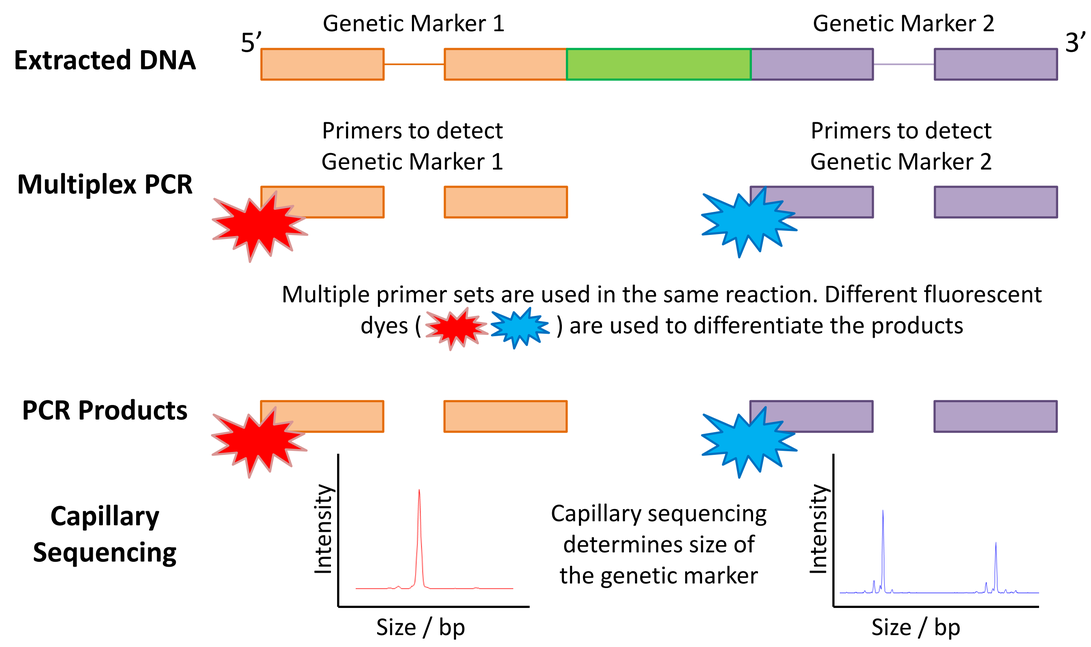
Sample collection

- 950 adult *F. hepatica* were collected from 44 naturally infected sheep
- 629 adult *F. hepatica* were collected from 31 naturally infected cattle

DNA Extraction

- DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit from the proximal end of the parasite (to avoid contamination with any eggs/sperm)

Genotyping



Statistical Analysis

GenClone 2.0 was used to calculate P_{sex} values to ensure any clones discovered were true clones and were not present due to chance.

Results and Discussion

Criteria	Sheep	Cattle	Sheep & Cattle
No. of animals	44	31	75
No. of parasites analysed	950	629	1579
No. of animals with clonal parasites	31[1]	15[1]	46
No. of clone sets (i.e. with the same genotype)	66	30	96
No. of clonal parasites	170[2]	81[2]	251
Proportion of clonal parasites	0.179	0.129	0.159

1 = no significant difference (X^2 : $P > 0.05$) 2 = significant difference (X^2: $P = 0.022$)
All P_{sex} values were significant <0.001

- The majority of animals (61%) contained clones. All the P_{sex} values were significant <0.001 so none of these clones were present due to chance
- The significant difference found between the number of clonal parasites in sheep and cattle may be because sheep tend to have higher parasite burdens than cattle
- The greatest number of clone sets in any one animal was 8
- The most parasites with the same genotype was 10
- Some of the clone sets were shared between animals: two pairs of sheep (from the same geographical areas) and two cows (from the same farm)
- The presence of clones within the definitive host most likely occurs following clonal expansion in the snail and aggregation of specific metacercariae on pasture which are then eaten by a sheep or a cow

Conclusion: The fact we have found parasites with the same genotype (clones) in our study means that specific genes, including those responsible for drug resistance, have the potential to spread through a population.