

Development of a pen-side diagnostic test for liver fluke infection in cattle and sheep

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Fasciola hepatica (the liver fluke)

- A common parasite of cattle and sheep in temperate climates
- Has a complex life cycle that involves an intermediate host, the mud snail, *Galba truncatula*
- It is highly pathogenic, and clinical signs include death, weight loss and anaemia
- Sub-clinical infections lead to significant production losses
- In cases of acute fasciolosis, farmers can lose up to 10% of their flock in a matter of days
- Prevalence of liver fluke is increasing significantly within the UK
- Evidence that resistance to flukicide drug, triclabendazole is increasing, particularly on sheep farms



Figure 1. A liver fluke

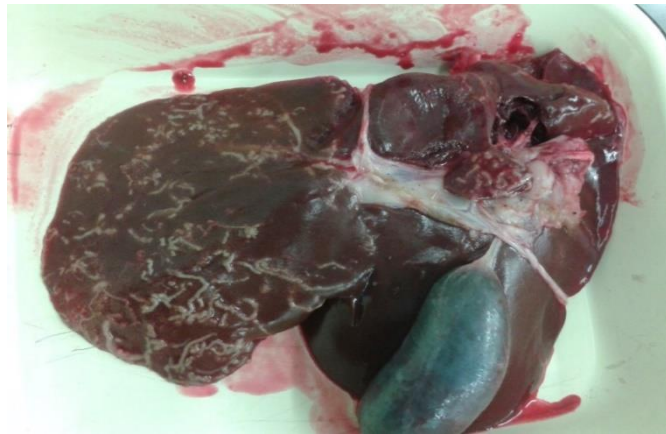


Figure 2. An infected liver

Current Diagnostics include:

- Faecal egg counts (FEC) lack sensitivity and only detect mature fluke infections, therefore cannot detect cases of acute fasciolosis
- The copro-antigen ELISA test detect fluke molecules in the faeces, can have similar sensitivities to the FEC in natural infections
- Antibody detection ELISAs which detect host anti-fluke antibodies in serum or milk samples; however these may detect historical as well as current infections
- For all available tests, samples have to be sent to the laboratory for testing which adds time and cost to the diagnosis



Figure 3. A liver fluke egg

Project Aims

- To produce a pen-side diagnostic test which could be used by farmers to help them make decisions on when to treat their animals

Objectives

- **Design a lateral flow test which could be used by farmers to diagnose fluke infection**
- First commercial lateral flow test was the human pregnancy test
- Using a blood from simple ear prick, this project aims to produce a rapid alternative to diagnose host anti-fluke antibodies in serum

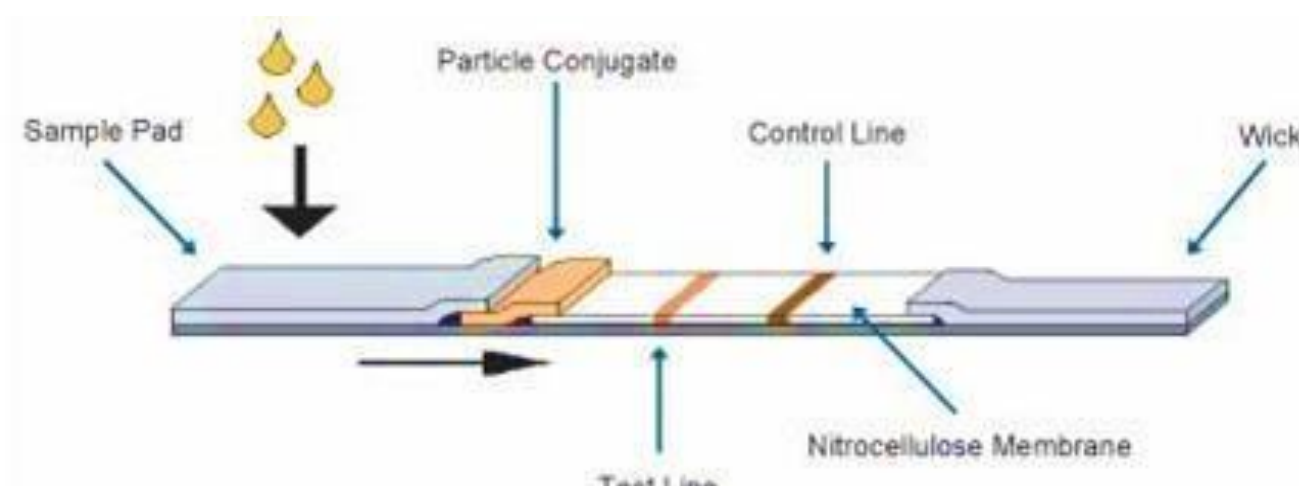


Figure 4. A diagram of a lateral flow test

Test Design: Test antigen

- Detection of circulating fluke antigens is difficult
- Test will aim to detect presence of anti-fluke antibodies in serum samples indicating exposure to infection
- Host antibodies recognise specific fluke molecules
- In order to produce a commercial test, need large amounts of the target antigen
- We showed that cathepsin L1 is the major fluke protein recognised by host antibodies
- A recombinant CL1 (rCL1) protein was produced by genetically cloning the gene for the protein into yeast to produce large amounts of rCL1
- In order to be used in a diagnostic test, recombinant antigen needs to be recognised by host anti-fluke antibodies

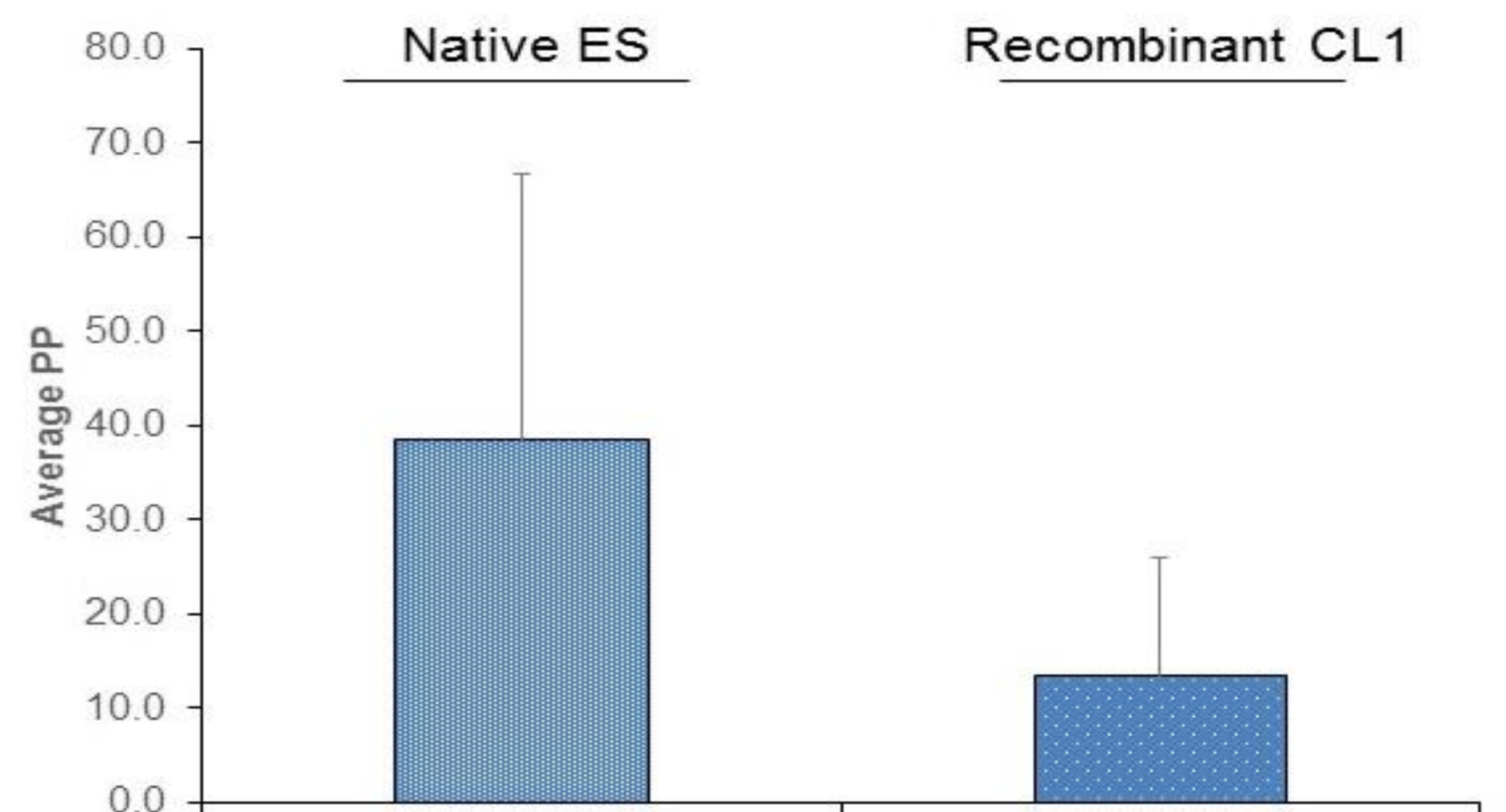


Figure 6. Shows the mean percent positivity of serum samples taken from naturally infected sheep tested against native and rCL1

- Naturally infected animals showed poor host antibody recognition to rCL1 compared to native fluke antigen
- Therefore native fluke antigen would perform much better in a diagnostic test

Final Design: Lateral flow test

- Designed and developed a prototype lateral flow test to detect host antibodies against fluke using native fluke antigen
- Test can be run in a matter of minutes
- Further validation is needed, before this test commercially available



Figure 6 Design and development of prototype lateral flow test

Conclusions

- Produced a recombinant version of the major fluke protein, CL1 in yeast, however this was not well recognised by host anti-antibodies from infected animals
- Successfully developed a prototype lateral flow test which can diagnose exposure to fluke infections in sheep and cattle in a matter of minutes