European Innovation Partnership (EIP) Wales

Fluke mapping using eDNA to inform the development of sustainable control measures

Final report

Operational Group:

Richard & Rosemary Tudor

Rhydian Evans

Einion Jenkins

Hefin & Jenny Evans

Hefin Hughes & Ruth Jenkins-Hughes

Dr Hefin Williams

Specialists:

Dr Rhys Jones (Researcher)

Phil Thomas (Vet)

Emma Jones - Facilitator (ADAS)

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Contents

Rumen and liver fluke in Wales

Rumen (*Calicophoron daubneyi*) and liver (*Fasciola hepatica*) fluke are parasites of agricultural concern in the UK. They affect a wide range of species; however, sheep and cattle are the main hosts in the UK. Both species of fluke share the same general life cycle and the same intermediate host, the mud snail (*Galba truncatula)* (Jones et al., 2015). Although rumen fluke is mainly reported in sub-tropical and tropical regions, its prevalence in the UK has been increasing in recent years (Huson, Oliver and Robinson, 2017). According to modelling, wetter, and warmer winters due to climate change are favourable to both parasites, which leads to a greater incidence of infestation over time (Fox et al., 2011; Caminade et al., 2015). Both parasites negatively impact animal health and welfare and have economic implications.

The two parasites have a similar life cycle (Figure 1). Infected animals excrete eggs within faeces, which hatch in water; juvenile flukes then infest mud snails which play a role in their development. Once sufficiently developed, juvenile flukes leave the host and infect definitive hosts (cattle or sheep) by encysting on the vegetation on which they graze. Once ingested, *F. hepatica* penetrates the intestinal wall and migrates to biliary ducts. *C. daubneyi* first damages the intestinal lining before migrating to the rumen.

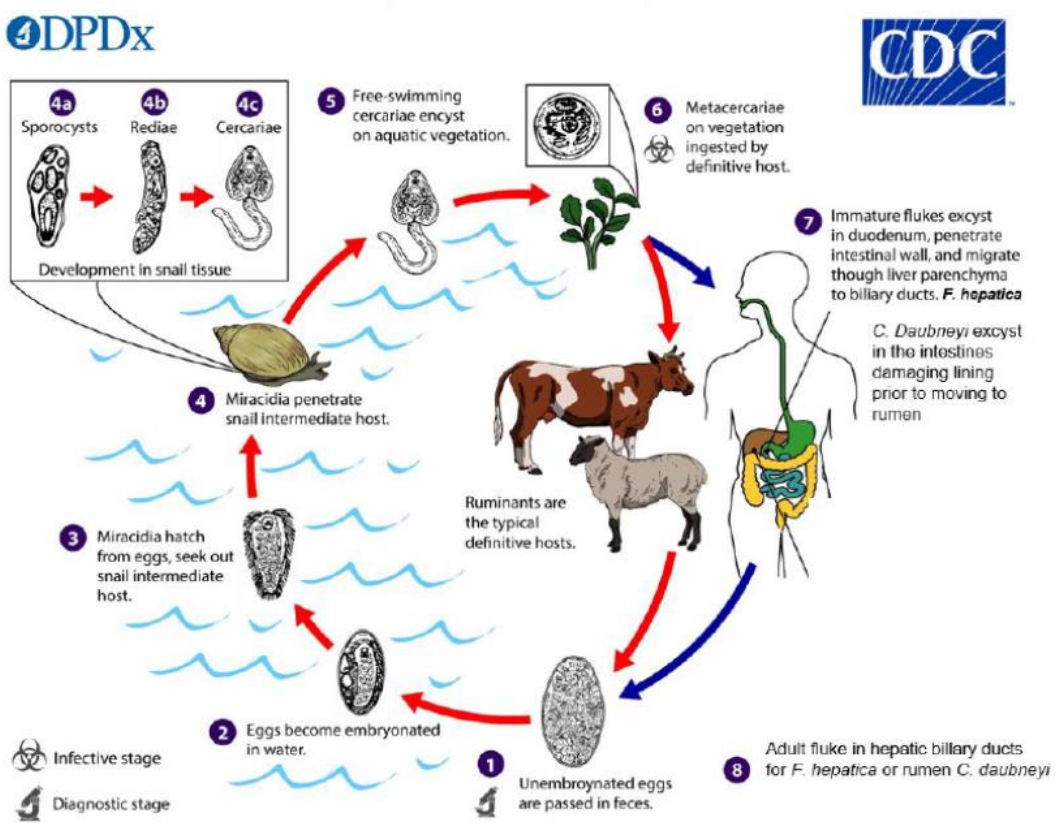


Figure 1: Life cycle of F. hepatica

The disease caused by *F. hepatica* is called Fasciolosis. It causes different symptoms depending on the infection stage. When juvenile flukes are ingested through the intestinal wall, the main symptoms are dyspepsia (bloating/discomfort) and ascites (fluid build-up in the abdomen). During the migration stage, these present as chronic/sub-acute symptoms including weight loss, body condition loss, poor fleece quality, lethargy, ventral oedema (bottle jaw) or acute symptoms such as reduced grazing and sudden deaths (Mitchell, 2002). As the flukes mature and reach the vicinity of the liver, symptoms are generally immune-inflammatory

linked such as fever, hepatomegaly, splenomegaly, and anaemia (Behm and Sangster, 1999). Unlike sheep, cattle require far higher chronic infection levels, and show clinical symptoms such as reduced milk yields, poor fertility, excessive weight loss, birthing weak calves and chronic diarrhoea (Howell et al., 2015).

The disease associated with the *C. daubneyi* infection is called paramphistomosis. It has been suggested to be a leading cause of livestock morbidity, causing symptoms including lethargy, severe scour, submandibular oedema, and dehydration during the immature parasite duodenal mucosa penetration (Tilling, 2013).

For both infections, the main diagnostic tool is faecal egg count (FEC) of mob faecal samples. Despite its efficiency, it can lead to blanket treatment, when only a handful of animals may have high fluke burdens (Radfar, Nourollahi-Fard and Mohammadyari, 2015). Due to the use of a shared diagnostic tool for both fluke species, misdiagnosis and treatment can occur, due to the similarity in the appearance of rumen and liver fluke eggs (Gordon et al., 2013). Treatment methodology on farms may include the use of a combination of products against fluke and nematodes. These are often applied at key times in the year and can lead to the off-targeted selection of resistances and negative influences on 'refugia' populations (Hodgkinson *et al.*, 2019). Such practices in the UK have led to the formation of 'industry-led' advice groups such as SCOPS (Sustainable Control of Parasites in Sheep). SCOPS encourages the informed, targeted use of anthelmintic treatment, following diagnostic confirmation (SCOPS, 2020).

Liver and rumen fluke have an impact on agriculture due to their effects on animal welfare and their economic implications for farmers and the industry. Mass treatments for *F. hepatica* alongside treatments for gastrointestinal nematodes are expensive and can lead to resistance issues. Each year liver fluke costs the UK cattle industry £23 million and around £3 to £5 per infected sheep (NADIS, 2016a, 2016b).

There is increasing interest in the implementation of targeted treatment management strategies based on individual diagnostics and defined thresholds (Charlier *et al.*, 2014; Calvete *et al.*, 2020). With high prevalence levels apparent across the UK, alongside evidence of increasing infection risk patterns, it is ever more important that new diagnosis and control strategies are put in place to combat this.

Development in the ability to screen environments using environmental DNA (eDNA) offers a unique opportunity to map infection risk down to specific field level within farm environments. Testing utilising eDNA provides positive or negative identification of snail/parasite risk areas and can be used to provide quantification of the level of infection risks in future. The ability to map risks down to field level can enable farmers to accurately implement informed strategies to reduce livestock and snail/parasite interactions and therefore reduce transmission rates.

Project Description

The project was a cooperation between a group of farmers from the Ceredigion area, IBERS (Aberystwyth University) and the Ystwyth Veterinary Practice in Aberystwyth. It was funded by the European Innovation Programme and ran from October 2020 to June 2022.

Project aims

The project aimed to investigate the use of field-level infection risk maps for both liver and rumen fluke based on eDNA detection to help control their prevalence on farms. Such maps would be developed to inform farmer decision-making around the management of fluke infection risk areas and livestock. Ultimately, the use of field-level maps allows farmers to make informed livestock management decisions, with their vets, that help with fluke control and reduce reliance on fluke control treatments.

Project design

The project was designed by Rhys Jones from IBERS and Philip Thomas from Ystwyth Vets, with facilitation provided by Emma Jones of ADAS.

The project ran from October 2020 to June 2022, with two periods of livestock and environmental sampling running from Autumn 2020 to Spring 2021 and from Autumn 2021 to Spring 2022. The two sampling periods enabled the farmers in the group to participate in testing and have discussions with IBERS and Ystwyth Vets to amend their management practices when required.

Livestock testing such as post-mortems, metabolic profiles and faecal egg counts were carried out to highlight any fluke burdens among the participating farms' livestock.

As previously explained, the project aimed to provide infection risk maps based on the detection of eDNA in water sampled across the farms to identify potential fluke infection areas. The areas were determined by combining Aberystwyth University's knowledge of favourable mud snail habitats, and the farmer knowledge of their own farms. The water that was sampled from the identified areas was then analysed to identify the prevalence and type of fluke on the farm.

Based on the results of the water sampling, field-level maps were then drawn for each farm highlighting the infection risk areas using the information gathered.

The farmers that took part in the project were involved with other Farming Connect initiatives.

The participants disseminated the messages from the project to their networks through several methods which included:

- existing Farming Connect (FC) initiatives which could link in with this project e.g., sheep discussion groups and study tours
- communicating with other FC and EIP-AGRI initiatives

During the project, the group produced factsheets, articles, and case studies in conjunction with Farming Connect, to share project findings with the wider industry. Timely project updates and information for press activity were provided to Menter a Busnes, Welsh Government and the EIP-AGRI network.

Methodology

The farmers who participated in the project were all clients of Ystwyth Vets who had been identified as having fluke burden issues on their farms. The following steps were all carried-out in 2021 and 2022.

Step 1: Identifying fluke burden on farm

To determine the potential fluke infection areas on each farm, knowledge of favourable mud snail habitats (called key habitat) and farmer knowledge of the land were used. The key, developed by Aberystwyth University, evaluated the suitability of sites for mud snail habitation based on soil type, moisture, shade, and grazing history.

Faecal egg count (FEC) of sampling groups of cattle and/or sheep or coproantigen ELISA (cELISA) testing were done to identify the prevalence and the type of fluke, on the farm. FEC is the most widely used diagnostic test as it is easy and cheap to perform. However, it can only detect the presence of adult parasites that are at least 10 to 12 weeks old. The cELISA test detects the secretion of antigens by the parasites in animal faeces, which allow for an earlier detection of fluke infestation. Post-mortem analyses were also carried out where there were sudden cattle and sheep deaths on the farms.

Metabolic profiles of the sampling groups were undertaken where appropriate to identify whether there was any underlying inflammatory disease burden or deficiencies of certain trace elements.

A method using environmental DNA was used to determine the presence of the infection vector organism, the mud-snail, within the areas selected. eDNA has previously been employed to confirm the presence of infection vector organisms within specific environments. eDNA has shown initial success in identifying agricultural bacteria that cause disease in crops and livestock. Environmental DNA and qualitative polymerase chain reaction (qPCR) based analysis techniques were used to map the prevalence of snails/fluke-infected snails on the sites.

Step 2: Sampling and analysis of eDNA

To avoid potential DNA contamination, the equipment was sterilised before use by soaking it overnight in 7.5% bleach. Disposable gloves were used for the collection of each separate sample.

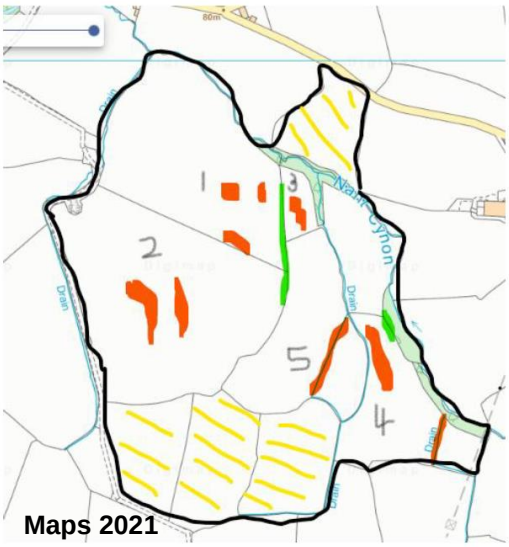
Sampling of eDNA consisted of taking 500ml of water from the identified sites (watercourses, poached areas on pasture or ponds). An average number of 11 samples were taken per farm. Then, the sample was filtered through 2.7micron glass microfibre filters. Pores within these filters captured eDNA whilst allowing small soil particles to pass through, thereby limiting clogging.

Samples were stored on ice and transported back to the laboratories in Aberystwyth within a day to avoid degradation of DNA post-collection. Samples were stored at -80*C until processing. DNA from the filters was extracted at IBERS using the Qiagen power soil extraction kit.

Step 3: qPCR Analysis and Mapping Methodology

DNA extracts were amplified using qPCR (quantabio Q) to identify mud snail eDNA. Qualitative PCR analysis determined if mud snails were present in a specific habitat and if there was evidence of liver fluke transmission.

Based on the results of these techniques, the farmers were provided with habitat maps of the areas where eDNA sampling took place on their farms. The maps showed fluke risks at a field-specific level and informed farmer decision making with regards to fluke management on farm. An example of the type of map provided is shown below:



Map of land at annotated with results of fluke survey. Red patches indicate approximate areas sampled which were positive for mud snail eDNA and thus are likely areas of fluke transmission on this farm. Green areas denote areas sampled that were negative for mud snail eDNA, however this does not confirm absence of mud snails. Yellow areas are fields that were not surveyed during visit.

Figure 4: Example of maps from eDNA testing

Step 4: Provision of advice and support to reduce fluke burden

Depending on the information collected on each farm, the farmers were advised by their vet and IBERS on ways to develop sustainable, cost-effective solutions to reduce fluke burden. Advice and treatment suggestions were tailored at farm level as the appropriate strategy for each farm varied.

The control strategies that were recommended to farmers depended on the risk areas, as well as the feasibility of the strategies on each farm. Some longer-term strategies were outside the scope of this project.

To gather wider information about fluke strategies and trends in fluke infection, Philip Skuce from Moredun Research and Jonathan King from the Wales Veterinary Service Centre (WVSC) presented to the farmer group in June 2022.

Results

Due to the differences in testing and timeline between the farms, results from each farm were treated separately.

Farm A:

Identified potential infection areas on the farm in 2021

Potential infection areas on the farm were identified by using the farmer's knowledge of the land and by looking for favourable snail habitat areas (open wet ground or bare mud). Pictures of sampled areas are provided below (Figure 3). Across the farm, fields contained multiple streams and/or ditches and wet and muddy areas that were ideal habitats for mud snails. It was noted that if fields were wetter in autumn, risk areas could expand.

Figure 5: Examples of risk areas on farm A

eDNA Mapping 2021 and 2022

In accordance with the results obtained from the sampling, a map of the risk areas was produced each year (Figure 4). The map created for 2021 sampling indicated that some of the areas sampled were positive for the presence of mud snails. The map created for sampling in 2022 focused on another location on the farm and showed that the main risk areas were found along the field boundaries.

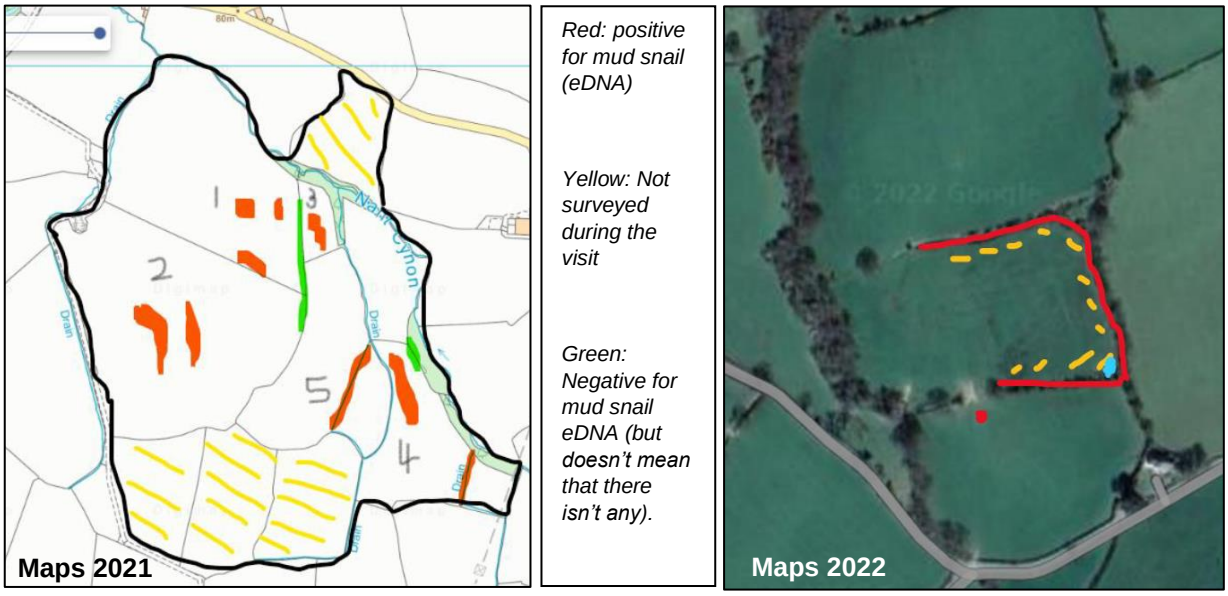


Figure 6: Farm A risk areas map

Livestock test results 2021

In 2021, Farm A had one faecal egg count done from bulked bovine faeces. The results are given in table 1 below. No liver fluke eggs were detected at the time and the parasite burden was low.

Table 1: Test results October 2021 Farm A

Faecal egg count & Copro-antigen results – October 2021			
Strongyle type eggs	Nematodirus battus eggs *(per g)*	Coccidial oocysts *(per g)*	Liver fluke eggs
Not seen	Not seen	200	Not detected

Recommendations 2021

Fencing off risk areas was not a suitable solution for Farm A as the habitats were present throughout the fields sampled. However, fencing off streams and ditches to prevent some contact between snails and livestock, financed by agri-environmental grants, was suggested. Failed drainage in field two created some snail habitats. Thus, fixing the drains would limit snail habitats in those areas. However, this was perceived to be costly.

Another recommendation was the regular testing of animals to identify if animals shed fluke eggs onto this land. Identifying clean land for a prolonged period over winter and testing and treating animals accordingly, before moving them to that land was a recommended strategy to reduce fluke egg contamination.

Livestock test results 2022

In 2022, further livestock tests were carried out. The results ar shown in Table 2. In January, FEC showed that rumen fluke eggs were detected in the faeces of ewes, while no eggs were found in yearlings and aged ewes. The overall parasite burden seemed to be higher in 2022 than in 2021.

Metabolic profile testing was carried out in February 2022 on six ewes, and it found that energy balance, protein status and mineral status were all acceptable. FEC testing of ewes in February 2022 showed that no liver fluke eggs were detected in faeces and no rumen fluke eggs were detected. One ewe was sent for post-mortem analysis in February 2022, liver or rumen fluke were not the cause of death.

Table 2: Test results 2022 Farm A

Faecal egg count – January 2022					
	Trichostrongyle type eggs *(per g)*	Nematodirus battus eggs *(per g)*	Coccidial oocysts *(per g)*	Liver fluke eggs	Rumen fluke eggs
Yearlings	200	150	1850	Not detected	Not detected
Ewes	500	<50	200	Not detected	**Detected**
Ages ewes	850	<50	100	Not detected	Not detected

Metabolic profile – February 2022		
Energy balance	Protein status	Mineral status
OK	OK	OK

Fluke egg detection – February 2022	
Rumen Fluke eggs	Liver Fluke eggs
Not detected	Not detected

Post-mortem analysis
No gastrointestinal disease of fluke

Farm specific strategies

General recommendations regarding housing, testing, treatment, and reduction of mud snail habitat are given in Appendix 1. Since the beginning of the project, the farm stopped purchasing cattle, hence eliminating one of the sources of fluke contamination by reducing the risk of buying in fluke-infected livestock.

Farm B:

Identified potential infection areas on the farm in 2021

Potential infection areas on the farm were identified through farmer knowledge of the land and by looking for favourable snail habitat areas (open wet ground or bare mud). Pictures of sampled areas are provided below (Figure 5). Many wet and muddy areas were identified in the fields.

Figure 7: Examples of risk areas on farm B

eDNA Mapping 2021

Based on the areas sampled around the farm, a map of risk areas was created. The map shows that the main risk areas on the farm seem to be the ditches in some of the fields. Unfortunately, some fields couldn't be sampled because of a lack of water but were considered

risk areas due to the boggy nature of the ground throughout the farm. No eDNA testing was carried out in 2022 as the parcel of land sampled in 2021 had been sold.

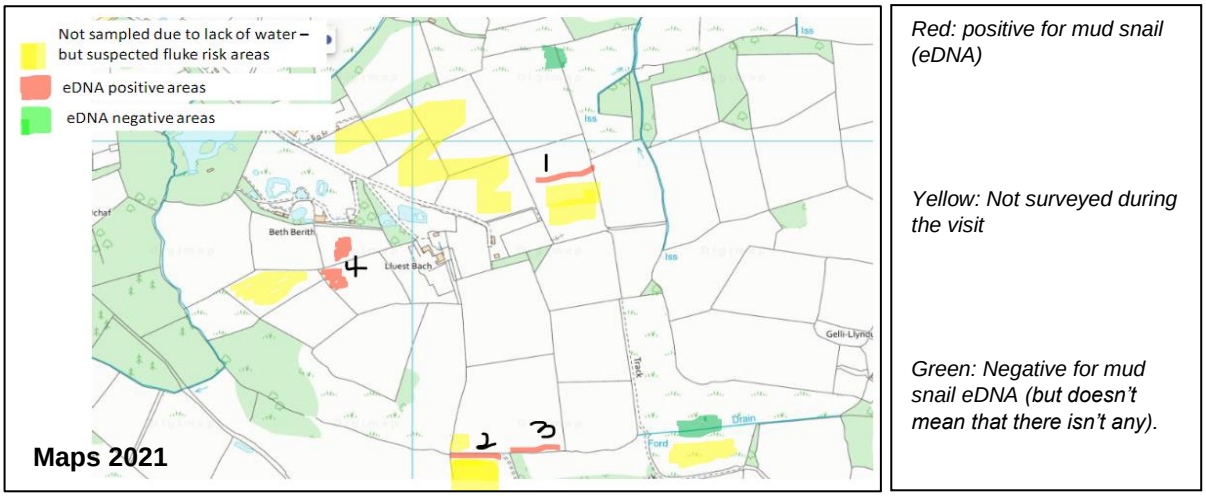


Figure 6: Farm C risk areas map

Livestock test results 2021

In 2021, a FEC test was carried out on bovine faeces to detect any liver or rumen fluke eggs. The test came back positive for rumen fluke eggs while it was negative for liver fluke.

Table 3: Test results October 2021 Farm B

Fluke egg detection – 2021	
Rumen Fluke eggs	Liver Fluke Eggs
Detected	Not detected

Recommendations 2021

For this farm, no recommendations were provided after eDNA sampling.

Livestock test results 2022

In February 2022, a metabolic profile was carried out on pregnant ewes. It showed that some of the ewes had poor long-term energy balance, which could negatively impact their health and productivity. Protein status and mineral status were found to be good.

A detailed FEC test was carried out in February 2022 on various batches of sheep and cattle. Like the year before, rumen fluke eggs were detected in both cattle and sheep. Only housed yearlings (sheep) did not test positive for fluke; however, this didn't mean they were not carrying immature fluke as well. The results are shown in Table 4.

Table 4: Test results 2022 Farm B

Metabolic profile – February 2022		
Energy balance	Protein status	Mineral status
Poor long-term balance	OK	OK
Fluke egg detection – February 2022		
	Rumen Fluke eggs	Liver Fluke eggs
Housed young bovine	**Detected**	Not detected
Housed adult bovine	**Detected**	Not detected
Housed ewes (triplet pen)	**Detected**	Not detected

Housed Ewes (thin singles and twins)	**Detected**	Not detected
Housed ewes	**Detected**	Not detected
Housed yearlings	Not detected	Not detected

Farm specific strategies

General recommendations regarding housing, testing, treatment, and reduction of mud snail habitat are given in Appendix 1.

Since the beginning of the project one potential source of fluke has been eliminated as a high-risk piece of land has been sold by the farm business.

Farm C:

Identified potential infection areas on the farm in 2021

Potential infection areas on the farm were identified by using farmer knowledge of the land and by looking for favourable snail habitat areas. Pictures of sampled areas are provided below (Figure 7). Boggy areas and many ditches were found throughout the farm. A ditch was previously fenced to limit access of livestock.

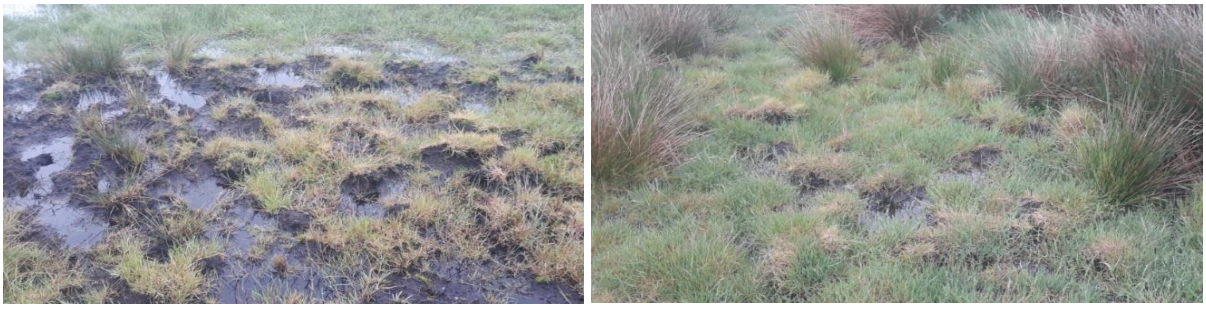


Figure 7: Examples of risk areas on farm C

eDNA Mapping 2021 and 2022

Based on the areas sampled around the farm, a map of risk areas was created (Figure 8). The 2021 map shows that the whole farm is considered as a risk area. This is due to the nature of

Red: positive for mud snail (eDNA)

Yellow: Not surveyed during the visit

Green: Negative for mud snail eDNA (but doesn't mean that there isn't any).

Figure 8: Farm B risk area maps

the wet and boggy ground around the farm, thus it was impossible to determine specific risk areas. The 2022 map showed that another piece of land, that wasn't tested in 2021 was a risk area as well.

Livestock test results 2021

In 2021, a FEC test was carried out on a batch of sheep and a post-mortem analysis (on a lamb) was done. Both rumen and liver fluke were detected in the flock. The post-mortem analysis concluded that the lamb died from acute pneumonia. Despite the high parasite burden, no links to either rumen or liver fluke were made.

Table 5 : Test results 2021 Farm C

Fluke egg detection – May 2021	
Rumen Fluke eggs	Liver Fluke Eggs
Detected	**Detected**
Post-mortem analysis – October 2021	
Post-mortem analysis of a lamb. Concluded that lamb died from acute pneumonia. Tests confirmed the presence of Mycoplasma Ovipneumoniae. The lamb had a high parasite burden with high FEC.	

Recommendations 2021

According to the test results and eDNA maps, recommendations were made to the farmer. In this case, fencing, draining, or rotational grazing were impossible. It was recommended to test and treat animals before the arrival of the livestock on the farm to limit egg shedding during early summer.

Livestock test results 2022

In February 2022, metabolic profile analysis was carried out on batches of suckler cows and ewes. Suckler cows had a good energy balance and protein status. However, they had low levels of magnesium. Both single and twin ewes had fair energy balance and mineral status. However, both batches had low protein status. This could highlight the presence of a long-term disease burden within the flock. The results are shown in Table 6.

Table 6: Test results 2022 Farm C

Metabolic profile – February 2022 – Suckler cows		
Energy balance	Protein status	Mineral status
OK	OK	Low in Magnesium
Metabolic profile – February 2022 - Ewes		
	Energy balance	Protein status	Mineral status
Singles	OK	Low	OK
Twins	Attention required closer to lambing time	Low	OK
Fluke egg detection – February 2022		
	Rumen Fluke eggs	Liver Fluke eggs
Suckler cows	**Detected**	**Detected**
Ewes	Not detected	Not detected

Farm specific strategies

General recommendations regarding housing, testing, treatment, and reduction of mud snail habitat are given in Appendix 1.

According to the results, mud snail DNA was found on both parcels of land tested. Liver fluke and rumen fluke was found in cattle faeces. The first piece of land sampled is part of a large, raised peat bog, therefore it will always be wet. It was recommended that cattle grazing there should be monitored for acute fluke during wet years.

It was also recommended to vaccinate pre turnout with a clostridial vaccine, to reduce the risk of black disease, and then to check three months later for the presence of eggs in faeces.

It was concluded that the rest of the second parcel of land isn't as likely to have fluke areas, but there were areas identified as being high risk in the eDNA mapping in 2022. In some fields the fluke risk could be more of a danger in dry seasons as the animals will tend to graze the wetter areas, e.g., where there are broken or blocked field drains.

Farm D:

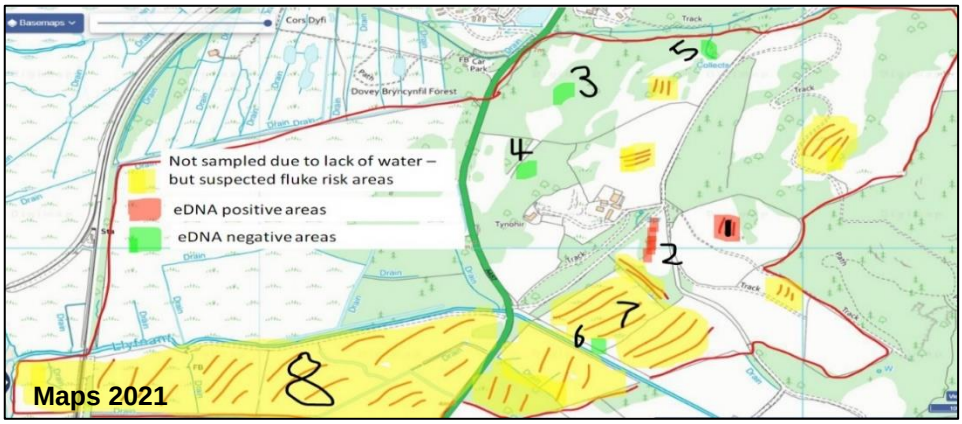
Identified potential infection areas on the farm in 2021

Potential infection areas on the farm were identified by farmer knowledge of the land and by looking for favourable snail habitat areas. Pictures of sampled areas are provided below (Figure 9). Multiple streams and wood areas were sampled across the farm. Fields 7 and 8 are known to be linked to historic fluke cases, thus they are now used only for cattle grazing and silage.

Figure 8: Examples of risk areas on farm D

eDNA Mapping 2021 and 2022

Maps were created according to the results obtained from the areas sampled across the farm (Figure 10). The map from 2021 showed that the main risk areas were located mainly across fields 6, 7 and 8. Positive areas were found in fields 1 and 2. However, no mud snail eDNA was found in fields 3, 4 and 5. Sampling in 2022 focused on the main risk areas identified the previous year, as shown in the second map below. The red circle highlighted on the second map shows that a positive area was identified in the fields below the A487.



Red: positive for mud snail (eDNA)

Yellow: Not surveyed during the visit

Green: Negative for mud snail eDNA (but doesn't mean that there isn't any).

Blue: Not sampled during visit due to lack of water but considered high risk

Figure 9: Farm D risk areas map

Livestock test results 2020

In November 2020, FEC testing was carried out and confirmed the presence of rumen fluke within the flock, A Coproantigen ELISA test was also carried out to further investigate the presence of liver fluke. The Coproantigen ELISA test provides earlier detection of fluke infestation. However, the ELISA test in this case was negative.

Table 7: Test results 2021 Farm D

Fluke egg detection – November 2020	
Rumen Fluke eggs	Liver Fluke eggs
Detected *(2 out of 6 animals)*	Not detected
Coproantigen ELISA - test liver fluke - November 2020	
Due to confirm negative egg detection test. Negative.	

Recommendations

As with other farms in the group, habitat fencing throughout the farm was not a feasible strategy. The farmer was encouraged to continue with the measures he was already using to reduce contamination, such as rotational grazing and testing and treating animals. It was also recommended to consider improving the drainage in field 1, which seemed to be a feasible measure.

Livestock test results 2021

The following year, in June 2021, a FEC test was carried out on a batch of ewes which showed that liver fluke eggs were present. A FEC test was carried out again on sheep in October 2021. It showed a high parasite burden, but no liver fluke. In November 2021, a FEC test was carried out on a batch of cattle as two bulk samples. One sample was positive for liver fluke, and one was positive for rumen fluke. The results are shown in Table 8.

Table 8: Test results 2022 Farm D

Fluke egg detection – June 2021				
Liver fluke eggs				
Detected				
Faecal egg count – October 2021				
Trichostrongyle type eggs *(per g)*	Nematodirus battus eggs *(per g)*	Coccidial oocysts *(per g)*	Strongyloides spp eggs	Liver fluke eggs
5,100	50	5,530	5,350	Not detected
Fluke egg detection – November 2021				
Liver fluke eggs		Rumen fluke eggs		
Detected *(1 out of 2 bulk samples)*		**Detected** *(1 out of 2 bulk samples)*		

Farm specific strategies

General recommendations regarding housing, testing, treatment, and reduction of mud snail habitat are given in Appendix 1.

It was concluded that the land near the river Dyfi will always be wet and so it was recommended to carefully consider timing of grazing this land, depending on rainfall. Additionally, if the land is grazed while it is wet, careful monitoring of fluke exposure through blood or faecal testing was advised to help keep on top of the situation.

The rest of the farm isn't as likely to have fluke issues, but it was recommended to be mindful of the areas highlighted by the snail survey as being high risk.

Farm E:

Identified potential infection areas on the farm in 2021

Potential infection areas on the farm were identified by using farmer knowledge of the land and by looking for likely snail habitat areas. Pictures of sampled areas are provided below (Figure 11). Ditches and boggy areas in fields 1 and 2 were identified as risks areas. Fields 3 and 4 had large boggy areas, which were favourable to mud snails.

Figure 10: Examples of risk areas on farm E

eDNA Mapping 2021 and 2022

Maps were created according to the results obtained from the areas sampled across the farm. The map from 2021 showed that the main risk areas were around ditches in fields 1 and 2 as well as across all of fields 3 and 4, as this was boggy ground. However, no mud snail habitats were found in field 5.

In 2022, the areas identified on the map were positive for mud snail eDNA.

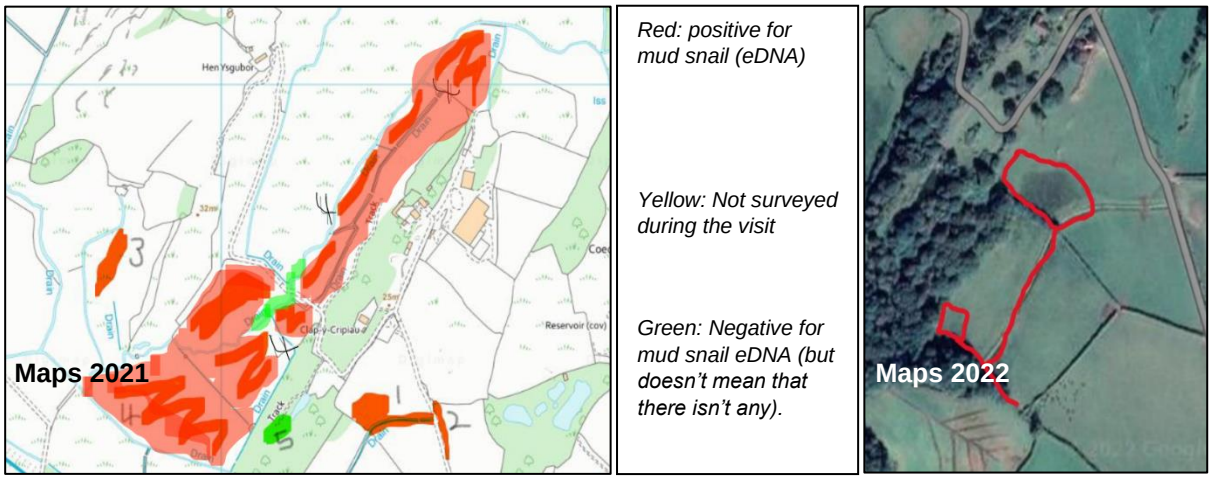


Figure 11 : Farm E risk areas map

Livestock test results 2021

One FEC test was carried out in February 2021 on a batch of cattle. It highlighted the presence of rumen fluke in the herd. No liver fluke eggs were detected.

Table 9: Test results 2021 Farm E

Fluke egg detection – February 2021	
Liver fluke eggs	Rumen fluke eggs
Not detected	**Detected**

Recommendations

Fencing or rotational grazing isn't a feasible option for this farm. However, fencing could be used to fence off streams and ditches to prevent contact between mud snails and livestock. The presence of positive areas in field 4 could be addressed by fixing failed drainage, although this may be expensive. It was also recommended to carry out regular testing of animals to identify if animals are shedding fluke eggs onto land, but this could be difficult due to the flexible housing policy on farm.

Livestock test results 2022

In February 2022, a FEC test was carried out for a batch of cattle. This identified the presence of rumen fluke in the herd, but no liver fluke.

Table 10: Test results 2022 Farm E

Fluke egg detection – February 2022	
Liver fluke eggs	Rumen fluke eggs
Not detected	**Detected**

Farm specific strategies

General recommendations regarding housing, testing, treatment, and reduction of mud snail habitat are given in Appendix 1.

It was concluded that the land near the river Dyfi will always be wet and so it was recommended to carefully consider timing of grazing this land, depending on rainfall.

The rest of the farm is likely to test positive for fluke, so it was advised to manage and monitor animals grazing areas identified as high risk.

It was also suggested to try to reduce the fluke risk in high-risk fields by using fencing to limit access to livestock to the stream at the edge of some fields.

Conclusions and Recommendations

Conclusions

The project confirmed the complex nature of liver and rumen fluke infection of livestock. In addition, the project confirmed that eDNA sampling could be used as a tool to identify high fluke risk areas on farm. This should be used alongside other strategies such as FEC and blood testing, implementing veterinary advice and treating livestock shortly after housing, before turnout and in early autumn in order to facilitate sustainable fluke management. Furthermore, as temperature and rainfall trends change, timing of fluke testing should also be varied accordingly.

Due to the short duration of the project and the Covid-19 restrictions which limited testing, farm visits and meetings in 2020 and 2021, it was not possible to carry out as much testing as was originally planned. Furthermore, after all testing was completed in 2021, the operational group identified that fencing off high fluke risk areas on the farms was not suitable in all situations. For example, fencing off rising springs or muddy tracks with small puddles was not practical or cost effective.

Minimising mud snail habitat and restricting access to these habitats by livestock is challenging. It can be costly or impractical depending on farm circumstances. An alternative approach which was adopted on some of the farms in the project was to test livestock and treat them accordingly before moving them to graze land where mud snails could be located. Essentially avoiding the introduction of liver fluke to mud snails, therefore preventing the completion of the fluke life cycle.

General recommendations

The following recommendations were highlighted during the project:

- Treat cattle and sheep shortly after housing to eliminate fluke, using flukicides suitable for use against immature and mature fluke (such as triclabendazole or closantel).
- To increase the likelihood of putting fluke-free cattle and/ or sheep on pasture, test faeces pre-turnout. If fluke eggs are detected in this test, treat animals with a flukicide which targets adult fluke (closantel or clorsulon).
- Test livestock in early autumn to decide if an autumn dose is needed. The aim is to reduce pasture reinfestation as much as possible as it will help controlling fluke long-term.
- Reduce mud snail environment to reduce the risk of summer infestation. This includes repairing drains and using vehicles that leave less ruts (wider tyres/ choice of route, etc).
- Limit the purchase/ introduction of animals from other farms and implement a robust quarantine procedure. This is also an efficient way to reduce fluke contamination risks.

Furthermore, it is important to consider changes in temperature and rainfall in the development of fluke management strategies as climate change has already lengthened the duration of the fluke season.

Recommendations for similar future projects

The project provided key learnings regarding the organization of such initiatives in Wales.

- The project provided the opportunity to implement findings from innovative research onto farms in practical terms. Further trials should be developed to to implement the approach on a wider scale and to consider cost effectiveness.
- Good project design and organization are essential to the success of this type of project.
- Sufficient administrative resource is required to support the project; for example, to contact, remind and support farmers to continue engagement with the project aims.
- Good communication is required to persuade farmers to take part. This project was aided by the fact that all the farmers in the group were Ystwyth Vets clients and were keen to address their fluke issues.

Appendix

Appendix 1: General recommendations / strategies against fluke

Fluke Plans / strategies	
Temperature and rainfall	A warm wet year is ideal for fluke. Once temperatures are over 8ºC both the pasture stages of the fluke lifecycle and snail activity increase. Changes in temperature and rainfall are also huge in the development of strategies – fluke season has already lengthened.
Rumen Fluke	Much is made of rumen fluke, but their importance is very debatable, some say they don't cause a problem unless there are huge numbers, others will say they are important.
Drug resistance	If drug resistance is detected on the farm, it is essential to not treat the animals with that product. Other products are available.
Type of land / favourable snail habitats	It is recommended to **reduce**, where possible, **the presence of snail habitats**. It will help fluke management by reducing the size/number of potential contamination risk areas. The main areas to look for are: - Broken/blocked drains - Tracks left by vehicles across fields - Muddy areas near the streams Where possible, it is recommended to: - to fence livestock away from those areas (often difficult to put in place on a farm) - to repair drains - to use rotational grazing - to move feed troughs and lick buckets - to use vehicles that leave less ruts
Purchase of animals	It is recommended to **limit the purchase** of animals, as buying in cattle is a potential to bring in large quantities of fluke and contamination of pastures with fluke and rumen fluke eggs.
Housing / treatments	Housing is a useful break from grazing and an opportunity to treat livestock against fluke. It is recommended to: - **Treat shortly after housing** – to clear any fluke being carried

	➔ Use of flukicide to kill mature as well as immature flukes (potential treatment options: triclabendazole or closantel) - **Test before turnout** – to detect if any eggs are present ➔ Use of adult flukicide (potential treatment option: oxyclozanide) - **Blood testing early autumn** - to indicate whether there needs to be an autumn dose needed For sheep flock, if no liver fluke eggs are found but rumen fluke is found, it is suggested to treat only the thinner ewes with oxyclozanide as the other will likely be OK.
Note	*The recommendations given below are general recommendations, thus they are not necessarily applicable to every farm.*

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