# European Innovation Partnership (EIP) Wales

## Is there something in the water?
## Identifying and addressing Cryptosporidium in sheep.

**Final Report**


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# EXECUTIVE SUMMARY


*Cryptosporidium* infects numerous species and negatively impacts animal health, productivity and can potentially affect human health. There are at least 45 different species of *Cryptosporidium*, with *C. parvum* being the major source of infection in lambs and calves. Transmission occurs through contaminated faeces either directly or indirectly (contaminated food or water). Research has shown that *Cryptosporidium* infections can negatively affect livestock health and impact farm economics. Moredun Research Institute (Scotland) showed that *C. parvum* infection can cause losses of £128 per calf[1]. Unfortunately, losses for the sheep industry due to *Cryptosporidium* are currently unknown.

The project aimed to improve the understanding of the persistence, transmission routes of *Cryptosporidium* on farms for the participating farmers and the wider industry. It also aimed to provide better knowledge of management options available on farms by giving insights on how pathogen burdens can be controlled on sheep farms.

The project ran from January 2020 to end of the summer 2022. Seven farmers actively participated during one sheep production cycle. Initial support, advice and guidance was provided by APHA (Animal and Plant Health Agency), HCC (Hybu Cig Cymru / Meat Promotion Wales) and DCWW (Dŵr Cymru Welsh Water). On-going project support was provided by Moredun Research Institute and Wales Veterinary Science Centre.

The project focused on assessing the presence of C*ryptosporidium* on farms through biosecurity reviews and testing of lambs and waterbodies at entry and exit points on the farm. The operational group (OG) worked together to develop procedures to reduce the prevalence of *Cryptosporidium* on farms. The project also investigated pathogen presence in other livestock and animals present on-farm other than sheep, and trialled novel cleaning methods, to gain farmer perspectives on improved hygiene protocols and their adoption.

The results from the project showed that *Cryptosporidium* was found in most waterbodies entering and leaving all farms. Six of the seven participating farms tested positive for *Cryptosporidium* when lamb faeces were sampled.

**A summary of project outputs:**

- Two farmer orientation meetings -one virtual, one face to face
- Three information dissemination meetings -one virtual and two face to face
- Regular OG/specialist project meetings -held virtually
- Seven farms in Wales took part in the study
- 2 – 7 visits per farm (median number of visits = 4) throughout the lambing season
- A total of 187 sheep samples were collected (average of 26.7 per farm, median of 24 per farm, with a range of 8 to 47 samples per farm)
- 17 samples from other species (cattle (n=8), dogs (n=4), rabbits (n=3), cats (n=1), pheasants (n=1)) were also collected
- Water samples were collected on four farms, three times: early March, mid-April, early June

The project contributed to a better understanding of the range of *Cryptosporidium* species on-farm and in water supplies and helped improve awareness of which species have an impact on human and livestock health.


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There was an increased appreciation in the OG of the benefits of reducing the incidence of *Cryptosporidium* in livestock; a twofold advantage- improving animal health and productivity and reducing contamination of the environment. This then has a positive impact on wider human health, reducing the risk of human exposure to *Cryptosporidium*.

The literature review undertaken out the outset of the project demonstrated that the general pathways of *Cryptosporidium* transmission are poorly understood, as are specific sheep related factors associated with the pathogen. The project has provided new information on the prevalence and sources of *Cryptosporidium* in sheep enterprises.

The project complements ongoing Moredun research into this subject area. It provides learning, which is being used to inform other *Cryptosporidium* in agriculture projects in Wales, working with Welsh Water.

The results of the study have also been presented at the 6th International Meeting on Apicomplexan Parasites in Farm Animals (ApicoWplexa 2022) at the Kongresszentrum Kreuz in Bern, Switzerland on the 6th of October 2022. The presentation was entitled: "Identification of Cryptosporidium spp. In livestock, domestic animals, wildlife and water source samples from seven farms in Wales".

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# 1 *CRYPTOSPORIDIUM* IN SHEEP

*Cryptosporidium* is a group of protozoan parasites which infect numerous species (cattle, sheep, goats, pigs, chickens, horses and deer). They negatively impact animal health, productivity and can potentially affect human health due to the zoonotic potential. There are at least 44 species of *Cryptosporidium* and more than 120 genotypes[2]. *C. hominis* and *C. parvum* are the cause of 90% of human infections, while *C. parvum* is the major source of infection in lambs and calves. *C. bovis*, *C. ryanae* and *C. andersoni* can also be found in cattle and *C. xiaoi* and *C. ubiquitum* in sheep.

In livestock, *Cryptosporidium* infections are mainly in young stock and can occur shortly after birth with key periods of infection between 1 to 3 weeks of age. Parasites are transmitted through faeces of the infected host either directly or indirectly (contaminated food or water supplies).

*C. parvum*, one of the main species causing infection in sheep, has a rapid life cycle and is transmitted when viable oocysts are ingested by susceptible hosts. Once ingested, the oocysts break open in the gut and release four infective parasites (sporozoites) which penetrate the cell lining of the small intestine. After developing below the cell membrane, oocysts are formed. Once mature, they are shed in the faeces as infective oocysts. Infection can reach high levels and spread rapidly. Usually, the cycle is completed in 3 to 4 days. However, autoinfection is possible, leading to longer (4 to 16 days) infection cycles. Under certain favourable circumstances such as cool, dark conditions, the tough outer shell of the oocyst means that it can remain dormant for up to a year in soil or low-turbidity water. The tough outer shell also makes the oocysts difficult to control as they are resistant to many commonly used farm disinfectants and water chlorination treatments.

*Cryptosporidium* infections can have significant animal health and economic impacts on livestock farming. Infected lambs are reluctant to suck while suffering from diarrhoea. In very young lambs, death can occur from dehydration. In poor weather conditions, lambs may die of hypothermia.

Research carried out by Moredun Research Institute in Scotland has found that *C. parvum* can cause significant financial losses for beef producers. Losses can reach £128 per a calf at sale[3]. Current statistics indicate it is the most common cause of scours in young calves, with symptoms ranging from mild scours to death of the calf. Unfortunately, the full scale of production losses to the sheep industry as a result of *Cryptosporidium* is unknown.

There is still a low level of understanding regarding the persistence, transmission routes, and management options available regarding *Cryptosporidium* in sheep. This project has contributed to a better understanding of the range of species on-farm and in water courses and helped improve awareness of which ones have an impact on human and livestock health.

Reducing the incidence of *Cryptosporidium* in livestock has a twofold advantage: improving animal health and productivity and reducing contamination of the environment. Thus, leading to lower human health risks. However, novel management solutions are required to eliminate *Cryptosporidium* from the environment. This project also investigated on-farm cleaning methods to reduce *Cryptosporidium* prevalence.

[2] Taxonomy and molecular epidemiology of Cryptosporidium and Giardia – a 50 year perspective (1971–2021), Una M. Ryan, Yaoyu Feng, Ronald Fayer, Lihua Xiao https://doi.org/10.1016/j.ijpara.2021.08.007

[3] Shaw HJ, Innes EA, Morrison LJ, Katzer F, Wells B. Long term production effects of clinical cryptosporidiosis in neonatal calves. International Journal for Parasitology (2020) 50: 371-376. DOI: 10.1016/j.ijpara.2020.03.002

# 2 PROJECT DESCRIPTION

## 2.1 European Innovation Partnership

This project was funded by the EU through the European Innovation Partnership (EIP) Wales programme, the role of which is 'to pool expertise and resources by bringing groups of people from different practical and scientific backgrounds together to tackle specific challenges, and trial new approaches which will be of value to others in the agricultural or forestry industry.' EIP Wales has received funding through the European Agricultural Fund for Rural Development and the Welsh Government.

In Wales, the implementation of the programme is managed by Menter a Busnes. Individual projects are managed and supported by 'Innovation Brokers', who are funded (separately and in addition to the EIP programme) through Farming Connect.

## 2.2 Project aims

The project aimed to provide better knowledge of prevalence, transmission routes, and management options available regarding *Cryptosporidium* in sheep. It also identified potential sources of infection and give insight into how parasite burdens can be controlled for the participating farmers but also the wider sheep industry. Additionally, the project provided knowledge to improve animal health and welfare and enhance wider ecosystem services provision through improvements in water quality.

## 2.3 Project design

The project ran from January 2020 to end of the summer 2022. Seven farmers actively participated in the project. Other members of the industry (including the APHA, independent vets, Moredun and Dŵr Cymru Welsh Water), participated in this project by providing support, advice and guidance during both during the development and the operational stages.

Originally the project was designed to follow four farms across two production cycles, with two farms carrying out in-depth testing. However, partly due to disruption associated with the COVID pandemic, project design was adapted with the approval of Menter a Busnes and Welsh Government.

The revised project carried out testing on seven farms over one production cycle. Water and infrastructure sampling was done on four of the seven farms by DCWW, while sampling of lambs from one week through to eight weeks in age occurred on all farms. The OG then came together to discuss results and management practices twice towards the end of the project, working with the project specialists.

Farmer participation was determined by DCWW's experience of working with members of the Penybont discussion group, in which several project farmers are active, and farmers identified by the two vet practices involved in the project -Hafren and Ddole Vets. DCWW helped with the identification of possible project participants, by funding lateral flow 'strip' tests for the vets to use with clients in the period running up to project operation. These tests helped identify flocks, which may be of risk of C*ryptosporidium* infection, as well as farmers who would be interested in further investigation/project participation.

The first step of the project was for all participating farms to undergo a biosecurity review and initial testing of watercourses prior to the lambing period to assess the risk level and presence of C*ryptosporidium* infection on the farms.

The second stage consisted of testing lambs on all farms during and after lambing. Sampling was undertaken on the same lambs at different stages in their development, between one and eight-weeks old.

Based on the sample results the OG worked on developing procedures to reduce the prevalence of C*ryptosporidium* on the farms, taking advice from Moredun Research Institute and the independent vet specialist, supplementing the advice provided by their own farm vet. Farmers and farm vets used this information to inform their animal health interventions, biosecurity/hygiene practices and water source management.

Other activities included assessing C*ryptosporidium* levels in the wildlife populations and other stock/animals on-farm and investigating different methods of cleaning to reduce the transmission of C*ryptosporidium* to other animals.

The project benefited from being able to send samples for testing to Moredun Research Institute and access expertise from Moredun to interpret results for farmers and provide guidance on practical control measures.

# 3 METHODOLOGY

## 3.1 Stage one: initial farm visit and biosecurity review

The first step consisted of reviewing the biosecurity on each farm during a visit from the farm's vet and the independent sheep vet. During that visit, vets discussed the project, and the issues farmers generally would face if/when *Cryptosporidium* was identified on their farms.

The biosecurity review and sampling protocol were co-designed by the vets. The reviews looked at all aspects of the farm environment and the biosecurity practices in place on the farms. This included quarantine procedures, cleaning and disinfection practices on farms. Testing of some ewes was also undertaken, to assess *Cryptosporidium* levels in the breeding flock at the start of lambing.

## 3.2 Stage two: sampling of watercourse and lambs

### 3.2.1 Watercourse sampling

An essential part of the project was to understand the prevalence of *Cryptosporidium* within the farm environment such as lambing sheds, bedding, and pasture. Any areas that were highlighted by the biosecurity reviews were particularly targeted during the sampling phase. Alongside the early stage of the project, DCWW separately funded strip tests for both Ddole and Hafren vets to use with clients who had issues with scouring lambs during 2020 and 2021, to help determine which farmers would benefit from participating in the EIP project.

According to research done by Moredun Research Institute, drinking water entering livestock sheds can be a source of *C. parvum* oocysts. Therefore, sampling was carried out in designated areas, (entry and exit points) around the farm buildings to establish the prevalence of *Cryptosporidium*.

A range of water course points were tested, for example:

- Water supply upstream of livestock
- Water testing of shed/field supply if on a private supply
- Spring water and borehole supply

### 3.2.2 Lamb sampling

The second stage of the project consisted of sampling lamb faeces from one week-old to two months old. In the first few weeks of life, lambs are most at risk to *Cryptosporidium* due to their lack of immunity, therefore samples of their faeces were taken during that period to monitor progression/prevalence. Individual lambs were selected based on their age at sampling and actual availability at the time of visiting the farm for sampling (based on what was convenient for the farmer).

The project initially had aspirations to sample both a control group and group exhibiting clinical signs on each farm, but it became clear that resource and engagement challenges for both the farmers and vets, at a very busy period in the farming calendar, made this unrealistic. Therefore, the methodology was modified to one that felt more achievable, and the project was also adapted to fund the time of a dedicated sampler, to ensure samples were taken as per the agreed timetable.

The farmer and sampler tried to ensure samples were taken from the same lambs at each sampling time points. Each batch was made up of four lambs and two batches were tested per farm. Lambs were tested each week between 1 to 8 weeks of age.

The sampling was undertaken by one or two individuals to assure consistence in sampling technique. As the sampling occurred during a high-stress period of the participating farmers, the sampling was done by an external contracted provider, supplied by ADAS.

### 3.2.3 Sampling of other animals on-farm

The project evolved during its lifetime to include additional investigation areas. These included testing for *Cryptosporidium* in other potential animal reservoirs across the farm, both in wildlife, pets and cattle.

### 3.2.4 Genotyping of Cryptosporidium Parvum

If samples tested positive for *Cryptosporidium*, Moredun then analysed these positive samples via genotyping, to ascertain more specifically what type of *Cryptosporidium* was present on the farms. It is worth explaining more about genotyping, as this was an important element to this investigative project (text provided Frank Katzer from Moredun).

Genotyping consists in identifying the different species of an organism. It can be used to study the diversify of a species, the origin of an organism or the ways an organism is transmitted. In *Cryptosporidium* investigations there are multiple tools that can be used to determine a source of an outbreak. The first stage is to determine which *Cryptosporidium* species are involved. Currently there are at least 45 recognised *Cryptosporidium* species and over 120 described isolates that are currently lacking sufficient biological data to determine if they are in fact separate species. Speciation is usually done by sequencing a relatively conserved gene, called the 18S small subunit ribosomal RNA gene (18S, for short). The sequence analysis of the 18S gene allows the identification of all described species and most of the described isolates. However, just determining species is often not sufficient to track a potential source of an infection or an outbreak and as a result more discriminatory methods are required that can determine variations within species.

In *Cryptosporidium* there are different genetic targets that have been used for sub-genotyping isolates. The most common and widespread method used for sub-genotyping *Cryptosporidium* focuses on a single variable gene called the *gp60* gene. The advantage of using this gene is that it has been used for a long time and there are many known reference sequences for different isolates and there is a reliable typing scheme in operation, however, the naming of the *Cryptosporidium* sub-genotypes, based on the *gp60* gene, is complicated.

Genotyping positive samples for *Cryptosporidium* is useful for determining the level of *Cryptosporidium* diversity within and between sheep farms, identifying potential sources of infection for lambs and investigating the potential role of water in the transmission.

## 3.3 Stage three: additional visits from independent sheep vet and local vet

As a result of the biosecurity review, water sampling and the sampling results of lambs tested, the OG worked together to develop procedures to reduce the prevalence of *Cryptosporidium* on the farms, and specifically *C. parvum*.

Recommended management options discussed in the OG included:

- Steam cleaning buildings to kill oocysts
- More frequent cleaning and disinfection of livestock sheds
- More frequent bedding down with straw
- Quarantine of scouring animals
- Ensuring that lambs receive adequate quantities of good quality colostrum, quickly.

The farmers and veterinarians provided feedback on the measures that they consider to be most effective. Advice and support from the veterinary expertise on the OG in conjunction with the veterinary service providers were essential in deciding on the bespoke treatment procedures for each farm.

In the case that *Cryptosporidium* was found on the farms, the farmers worked with the veterinarians to identify the source and treat it immediately.

## 3.4 Stage four: trailing cleaning methods

Cleaning techniques were trialled as part of assessing different options for *Cryptosporidium* and wider health management issues during lambing. Although investigating hygiene techniques was a general objective of the project, specifically trialling more novel methods for the sector -i.e., hiring kit for use on-farms- was not costed in the original proposal.

OG members became interested in learning more about steam cleaning, UV cleaning and hydrogen peroxide cleaning. However, due to issues in finding a supplier within the timescale of the project, trialling UV and hydrogen peroxide cleaning were not possible. It became clear that UV cleaning is very specialised and the use of it in beef housing and sheep lambing sheds isn't common or appropriate. The shed infrastructure isn't necessarily suitable as it was not originally set up for such cleaning methods, unlike poultry sheds (for example not all lambing sheds have hard, washable surfaces).

Instead, two cleaning methods were trialled in the later stages of the project:

- Steam cleaning + disinfectant,
- Cold pressure washing + disinfectant.

## 3.5 Sharing and dissemination of information between OG members

The results of the project were shared and discussed on an ongoing basis with the group via virtual video calls, as well as two face-to-face meetings.

This project development and information sharing process was also particularly important for DCWW -the industry partner- who was interested in:

- the prevalence of use and effectiveness of biosecurity measures
- where the parasite may be present across farm water supplies
- what the financial impact of the parasite is in sheep and
- any learnings as regards messages to the wider industry on the value of reducing pathogen incidence and spread for livestock and human health.

Moredun worked with the group providing technical support at two evening farmer meetings, and ongoing support on virtual calls, such as results interpretation and helping all participants develop a broader understanding of the topic.

# 4 RESULTS

## 4.1 Farm participant characteristics

Seven farms took part in the project. Of these seven, four farmers agreed to the sampling of their water supplies.

Six farmers have beef cattle as well as sheep; one participant is a sheep only enterprise.

Farms' flock size varied between farms, with the number of breeding ewes ranging from 115 to 1,750.

All the farms buy in stock, as is the practice for most sheep producers. Six participants buy in rams and some ewe replacements, while one farm only buys rams in, and only uses home reared ewe replacements.

Grazing systems are different across the participants. 28% of the participating farms have multiple holdings and 57% have the use of winter keep. 43% of the farms use open hill-grazing, shared with neighbouring farms. Over 80% of the farmers co-graze sheep and cattle.

## 4.2 Biosecurity review information

As outlined in figure 1, 57% of the farmers have species-specific sheds (i.e., designated cattle, or sheep sheds). Most participants share management equipment across their sheep and cattle enterprises, and this equipment is usually disinfected.

The reviews evidenced that quarantine procedures vary in stringency and methodology across the farms.

One farm does not treat or use quarantine methods at all.

One farmer does not quarantine rams at all and is of the opinion that pathogen risk is low, as all rams are bought from the same source each year.

On the remaining five farms at least three weeks quarantine is carried out along with some treatment (fluke, worming).

Only one farm quarantines purchased stock for three to four months, treats them for fluke, worms and also treats for scab pre-sale.

All farms had no visitors or a very limited number of personnel on the farm (part-time workers or contractors). One farm reported a case of *Cryptosporidium* in a family member or worker in the past.

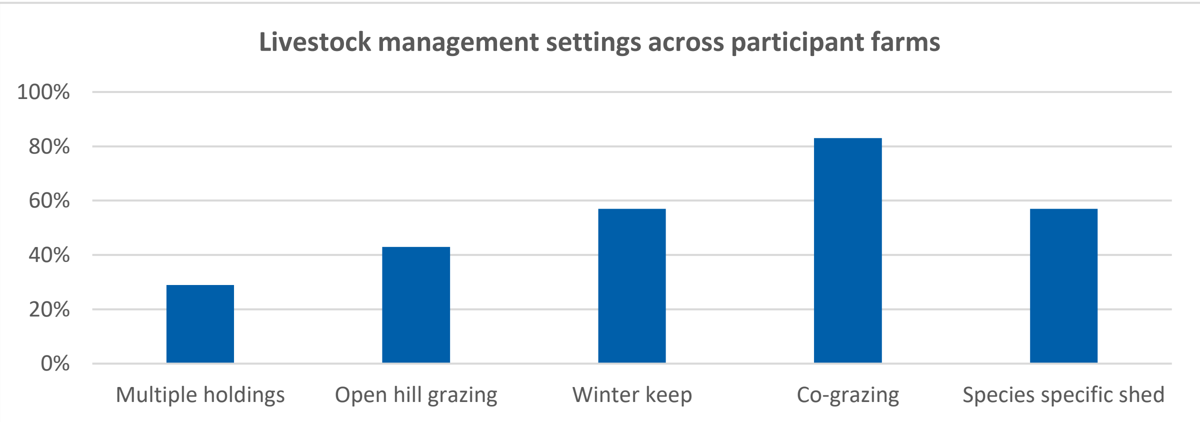


**Figure 1: Livestock management settings**

Three out of seven farmers disinfected waterproofs, hands and/or equipment (e.g., buckets) before handling other animals.

Regarding exterior potential *Cryptosporidium* sources, wildlife sources cited included pheasants, rabbits, deer, voles, rats. Feed storage and feed distribution control methods were followed to reduce contamination as much as possible by storing in bins or tote bags, secured from wildlife.

A range of livestock drinking water sources are present across the farms. Four have access to a stream or river, while most farms have access to a borehole or well for the sheds. Only two farms use mains water as a water source for either fields or housing shed. Two farms had their water sources tested in the previous year, prior to the project, with no contamination from *E. coli* or C*ryptosporidium* found.

Muck was stored for 3-16 months, with most of the farms storing manure for around six months. Farmers reported to wait at least six weeks to eight months before letting livestock graze on the fields after spreading manure.

Indoor lambing protocols vary between the farms. Whilst six out of seven use disinfectant, only one of the farms disinfects pre-lambing, the others only use lime pre-lambing.

Only three farmers use lime between change overs in individual pens.

On most farms, scouring lambs are isolated and equipment disinfected.

Two of the seven farmers reported wearing gloves when in the shed.

Before being turned out, lambs are castrated (5 farms) and tail ringed (6 farms). Generally, new-born lambs and ewes move to fields near the buildings, divided into age groups and the ewes are fed for a minimum of 4 weeks after lambing.

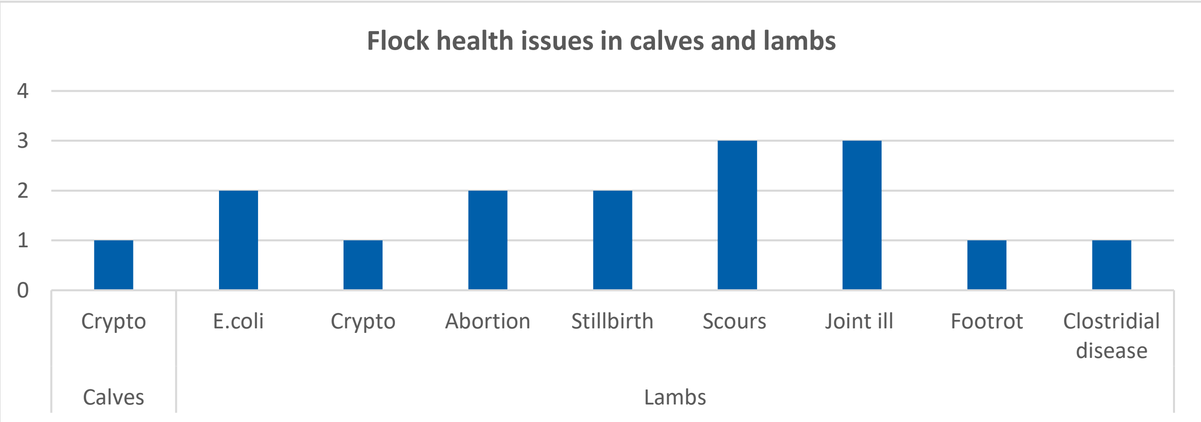


**Figure 2: Flock health issues observed in calves and lambs**

Different flock health issues were reported across all farms in the biosecurity reviews (figure 2).

Only two of the farms had *Cryptosporidium* diagnosed in the past. Three farms had health issues due to *E. coli*, stillbirths and lambs that presented scouring. Four farms reported joint ill in lambs. Occasional footrot, clostridial diseases and scab infection were also reported.

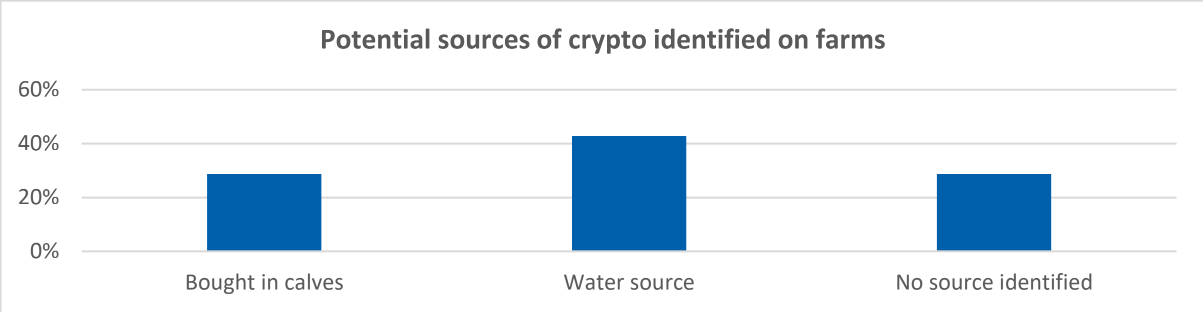


**Figure 3: Potential sources of Cryptosporidium identified by the farmers**

As part of the biosecurity review stage of the project farmers and their vet identified different potential sources of *Cryptosporidium* on their farms (figure 3).

Over 40% of them believed that their water source is a potential source, while 28% consider bought-in calves were a potential source. Another 28% of the farmers did not identify any source of *Cryptosporidium* on their farm, either based on their understanding of their own farm health status, which may or may not be backed up by testing.

Control measures to limit contamination relied on the use of disinfectants and using testing (bloods and faecal samples), but the use of these tests was not routine. Another control measure stated was the avoidance of buying in young calves.

# 5 CRYPTOSPORIDIUM TESTING DURING THE PROJECT - RESULTS

Moredun provided specialist services into the project, analysing water and faecal samples, to identify presence and type of *Cryptosporidium* on the participating farms. The results of the samples analysed are outlined in this section.

## 5.1 Watercourse sampling

Four of the participating farms had watercourse sampling done, undertaken and paid for by DCWW. The sampling consisted of testing water sources either entering or leaving the farm at three different periods. A description of the sampled streams or water sources is given for each farm on figure 4. Details of the results obtained for each farm is given in table 1.

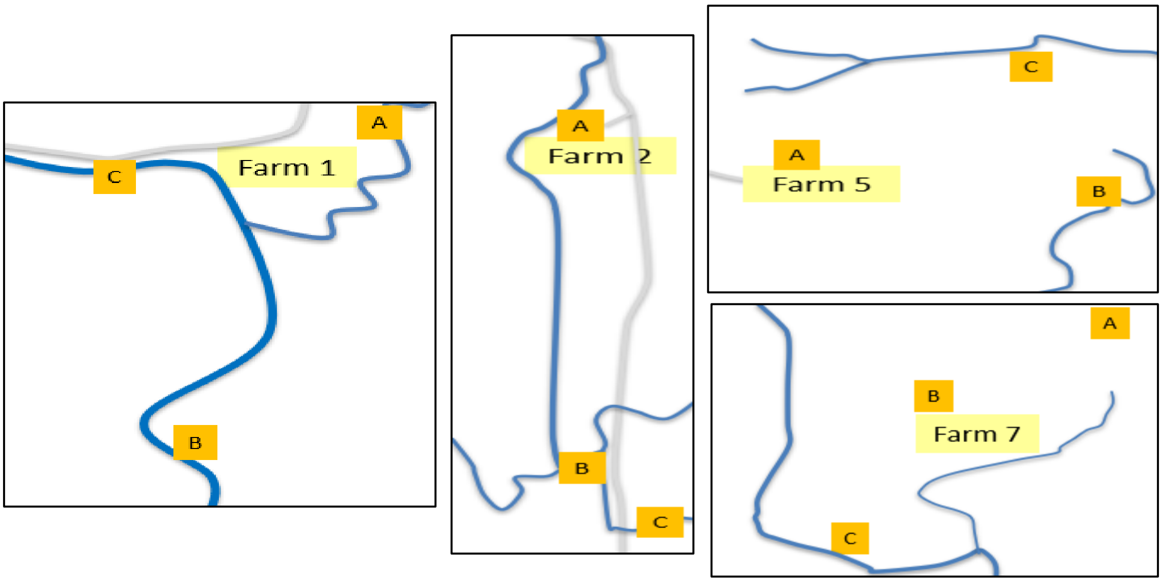


**Figure 4: Water sampling points entering and leaving the farms**

The results of the testing showed that:

At farm 1, tests confirmed the presence of *Cryptosporidium* (*C. parvum, C. andersoni*) both entering and leaving the farm.

At farm 2, the water source (tap in yard) was free from *Cryptosporidium* while the river and stream close to the farm were *Cryptosporidium* positive (*C. parvum, C. andersoni*).

At farm 5 water sources (tap in shed and spring source) tested positive only during the first visit (in March), while the river tested positive at all visits (March, April and June).

At farm 7, a new bore hole water tank tested positive at the first visit in March. However, this could have been due to the borehole not being sealed when established, which caused its contamination with *Cryptosporidium*. Both other points sampled came back positive for *Cryptosporidium* (*C. parvum* + *C. andersoni*) during the other visits.

**Table 1: Results from water sampling**

| Farm 1 | 1st Visit (March) | 2nd Visit (April) | 3rd Visit (May) | Farm 2 | 1st Visit (March) | 2nd Visit (April) | 3rd Visit (May) |
|---|---|---|---|---|---|---|---|
| A (River entering farm) | ***C. parvum*** + *C. andersoni* | ***C. parvum*** + *C. andersoni* + *C. ubiquitum* | ***C. parvum*** + *C. andersoni* | A (tap in yard) | Negative | Negative | Negative |
| B (River leaving farm) | ***C. parvum*** + *C. andersoni* | ***C. parvum*** & *C. proventriculi* | ***C. parvum*** + *C. andersoni* | B (river) | ***C. parvum*** | ***C. parvum*** + *C. andersoni* | ***C. parvum*** + *C. andersoni* |
| C (River leaving farm) | ***C. parvum*** + *C. andersoni* | *C. andersoni* | *Not done* | C (stream) | ***C. parvum*** + *C. andersoni* | Negative | ***C. parvum*** |

| Farm 5 | 1st Visit (March) | 2nd Visit (April) | 3rd Visit (May) | Farm 7 | 1st Visit (March) | 2nd Visit (April) | 3rd Visit (May) |
|---|---|---|---|---|---|---|---|
| A (tap in shed) | ***C. parvum*** | Negative | Negative | A (New bore hole tank) | ***C. parvum*** | Negative | Negative |
| B (River) | ***C. parvum*** + *C. andersoni* | ***C. parvum*** + *C. andersoni* | ***C. parvum*** + *C. andersoni* | B (Spring source) | *C. canis* | ***C. parvum*** + *C. andersoni* | Not done |
| C (Spring source) | *C. parvum* | Negative | Negative | C (River - cattle drinking point) | Not done | ***C. parvum*** + *C. andersoni* | ***C. parvum*** + *C. andersoni* |

## 5.2 Sampling of lambs

### 5.2.1 *Cryptosporidium* testing

All farms tested positive for *Cryptosporidium* in lambs for at least one of the sampling points across the 1 to 8 weeks of sampling, and it was also found in some adult animals on some farms (figure 5).

However, not each farm tested positive at each age (figure 6). *C. parvum* was found in sampled animals at one, two, three, five, six, seven-week-old lambs and adult animals. The number of positive cases was the highest at week three and four and the lowest at week five. However, the number of adult ewes sampled, testing positive was high.

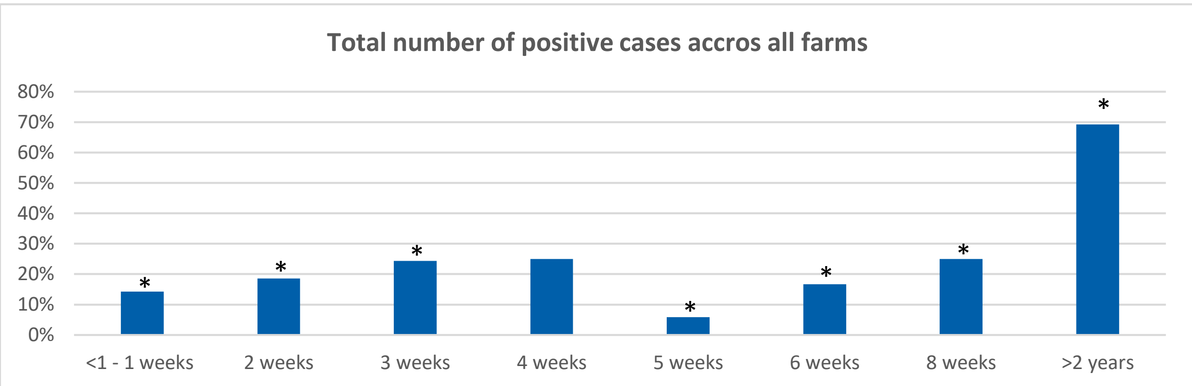


**Figure 5: Total number of positive cases of *Cryptosporidium***

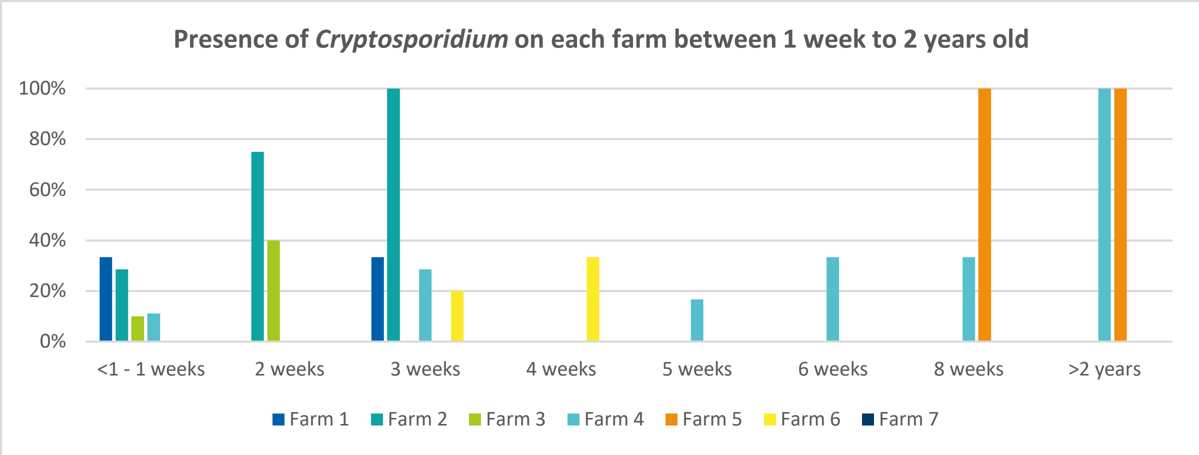


**Figure 6: Presence of *Cryptosporidium* in one week to eight-week-old lambs and adult ewes for each farm**

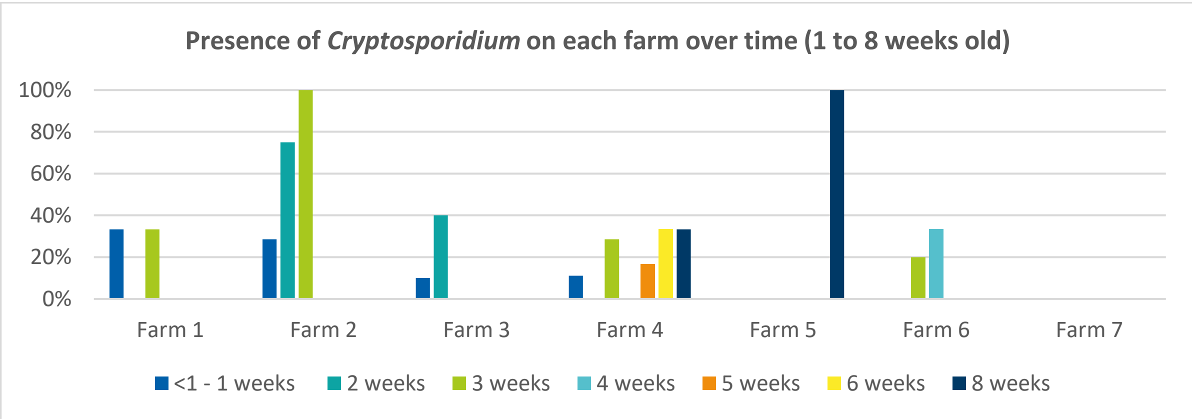


**Figure 7: Evolution of the presence of *Cryptosporidium* on each farm in one week to eight-week-old animals**

When focusing on the evolution of the prevalence of *Cryptosporidium* over time, figure 7 shows that the presence of *Cryptosporidium* varied.

Farms 1,2, 3 and 6 had lambs testing positive only during the first four weeks, while farm 5 had positive results only at 8 weeks of age.

Unlike the other farms, lambs from farm 4 tested positive nearly each week.

Additionally, out of the thirteen samples tested from other species (6 calves, 4 dogs, 3 rabbits, 2 cows, 1 cat & 1 pheasant), six were positive (4 calves, 1 dog & 1 rabbit).

### 5.2.2 Genotyping of positive tests

If samples tested positive for *Cryptosporidium*, Moredun then analysed these positive samples further using genotyping. The benefits of genotyping *Cryptosporidium parvum* positive samples are as follows:

1) Determine the level of *Cryptosporidium parvum* diversity within and between sheep farms.
2) Identify potential sources of *Cryptosporidium parvum* infection for lambs.

3) Investigate the potential role of water in the transmission of *Cryptosporidium parvum*.

### 5.2.3 *Cryptosporidium parvum* diversity levels within the project's farms

In total, 39 sheep tested positive for *C. parvum* and the *gp60* genotype was successfully determined for 27 lambs, aged between 1 and 8 weeks of age, and also in 4 sheep that were 2 to 4 years of age. The lambs originated from five different farms and the adult sheep were from two of these farms.

The *gp60* sub-genotypes for all these were all identical to each other and they were **IIaA15G2R1**. The IIaA15G2R1 genotype is the most common *Cryptosporidium parvum gp60* sub-genotype within Europe and North America.

As a result, this study did not reveal any differences in *Cryptosporidium* genotypes between sheep of different ages and no differences in genotypes between the farms.

Twenty one of the 35 water samples that were tested from four of the seven farms contained *Cryptosporidium parvum*. Genotyping was successful for 12 of these samples, revealing that the IIaA15G2R1 *gp60* sub-genotype was most common (10/12 samples) and present in water samples from all four farms.

Two farms also had one water sample each that contained the IIaA16G2R1 *gp60* sub-genotype, which was not detected in any of the animal faeces.

It cannot be ruled out that the water on the farms was a source of *Cryptosporidium parvum* infection for the lambs because the IIaA15G2R1 *gp60* sub-genotype was so frequently found in the water samples as well as in the lambs.

However, it can also not be ruled out that the *Cryptosporidium parvum* oocysts from the animals on the farm did not contribute to the contamination of the water courses running through the farms.

The same *gp60* sub-genotype (IIaA15G2R1) was found in all *Cryptosporidium parvum* positive sheep samples as well as in two calf samples and ten water samples on four farms.

When looking at the other host species that were tested, the *gp60* sub-genotype could only be determined for three calf samples from two farms, where they had *Cryptosporidium* in their lambs (Farm 1 and Farm 2).

On farm 2, two of the calves had the same *gp60* sub-genotypes, IIaA15G2R1, as the lambs.

However, on Farm 1 the positive calf had the IIaA13G2R1 sub-genotype, while the lambs on that farm had the IIaA15G2R1 sub-genotype.

One dog and one rabbit sample were *C. parvum* positive but unfortunately no *gp60* sub-genotype could be determined for these samples. Additionally, the same *gp60* sub-genotype (IIaA15G2R1) was found in all *Cryptosporidium* positive sheep samples as well as in two calf samples and ten water samples on four farms.

Unfortunately, from the data available from this study, it cannot be concluded whether dogs, rabbits, pheasants or cats were potential sources of *Cryptosporidium* for the lambs or not.

### 5.2.4 Moredun conclusions from the sample results

One of the main limitations of the study is that the *gp60* sub-genotyping method did not have enough discriminatory power to distinguish between most of the *Cryptosporidium parvum* positive samples.

It is unfortunate that all the sheep had the most common *gp60* sub-genotype (IIaA15G2R1), which reduced the level of information we could obtain about the spread of the infections within the flocks that were investigated.

Genotyping is further compounded by the fact that each parasite has only a single copy of the *gp60* gene, which reduces the sensitivity for this marker in comparison with the 18S locus that is used for species determination, which has multiple copies within each parasite.

An additional limitation is using only a single DNA target for genotyping, ideally several DNA targets would be typed to gain more discriminatory power, but that significantly increases time/costs of the genotyping and unfortunately, there are no other markers that are as well characterised as the *gp60* gene.

The same *gp*60 sub-genotype (IIaA15G2R1) was found in all *Cryptosporidium parvum* positive sheep samples as well as in two calf samples and ten water samples on four farms.

Therefore, it cannot be ruled out that lambs became infected with *Cryptosporidium parvum* either from adult sheep, calves or their water supplies.

Both the water and the faecal sample results help to underline how challenging ascertaining the source infection points is and the likely cycling of the pathogen between livestock and water supplies. This underpins the importance of biosecurity and hygiene practices, benefitting all livestock production, and minimising pathogen spread around the system, reducing both livestock and human exposure.

## 5.3 Cross referencing biosecurity review detail with water and lamb sampling results

All four farms that had water sampling carried out had good biosecurity in place on their farm with most of the boundaries double fenced, separation of bought-in animals, shed cleaning protocols, change/use of clean bedding and disinfection of pens at changeover during lambing.

None of the farms reported an issue with *Cryptosporidium* at the start of the project, but the testing showed that strains of *Cryptosporidium* were present in both the water supplies and in livestock.

Private water supplies (private tank, bore hole) tested had a lower incidence of *Cryptosporidium* than water courses (i.e., stream, spring and rivers).

*Cryptosporidium* was detected in lamb samples at three of the four water sampled farms. Two of the farms had positive results for young lambs (1 to 3 weeks of age), while on the third farm *Cryptosporidium* was detected only in 8-week-old lambs and adult ewes.

Table 2 provides a comparison of the biosecurity features of each water sampled farm, alongside their water and livestock faecal sample results.

**Table 2: Summary of biosecurity reviews, water and lamb sampling results**

| | Farm 1 | Farm 2 | Farm 5 | Farm 7 |
|---|---|---|---|---|
| Bio security review | - 90% boundaries double fenced<br>- Bought in ewes and rams separated for 3 to 4 months + wormed, fluke dipped, and scab treated.<br>- No co-grazing during lambing<br>- Same sheds for cattle and sheep – disinfected and washed between species<br>- Most fields have access to river<br>- 2 to 3 fields and sheds are on main<br>- All sheds cleaned and disinfected pre-lambing<br>- No identification of potential crypto<br>- Currently no measure to prevent *Cryptosporidium* | - 85% of boundaries double fenced<br>- Bought in ewes and rams separated for 3 to 4 weeks<br>- No co-grazing Usually shed species specific<br>- 60 to 70% water from river<br>- Spring well used for sheds<br>- Lime daily between each ewe. Shed pressure washed and disinfected<br>- Scouring calves 5 to 6 years ago – *E. coli* detected<br>- Measure to control *Cryptosporidium*: disinfect as much as possible | - Boundaries double fenced<br>- Bought in ewes and rams separated for 21 days<br>- Shed species specific<br>- No co-grazing<br>- Water from tanks mostly<br>- Water in sheds from wells<br>- Individual pens disinfected between use<br>- Powered disinfectant used. Lambing equipment disinfected.<br>- *E. coli* confirmed in lambs before<br>- Borehole potential source<br>- May fence off stream | - Boundaries double fenced<br>- Bought in rams separated for 7 days and wormed<br>- *Cryptosporidium* and rumen fluke detected in cattle<br>- Occasional co-grazing<br>- Shed species specific<br>- Main waterbodies: borehole, water tanks<br>- Lime applied to the floor and deep fresh litter regularly Individual pens drenched with iodine and clean fresh bedding<br>- Watercourse likely to be a source of *Cryptosporidium*<br>- No issues in lambs, occasional random faecal test. |
| Water testing | *C. parvum* detected in waterbodies entering and leaving the farm | *C. parvum* detected in river and stream. Not detected tap in yard (spring source). | *C. parvum* detected mainly in river. Detected in borehole and spring only during 1st visit | *C. parvum* detected in new bore hole tank, spring and river during different visits. |
| Lamb testing | *Cryptosporidium* detected weeks 1 and 3 | *Cryptosporidium* detected weeks 1 to 3 – peaking at week 3 | *Cryptosporidium* detected week 8 and over 2 years | No *Cryptosporidium* detected |

## 5.4 Cleaning methods results

Based on the farmer feedback from cleaning method trialling, the use of both cold pressure washing and steam cleaning was advantageous and provided increased farmer confidence in the cleanliness of the shed.

For steam cleaning, the sheds were first pressure washed, steam cleaned and then disinfected which took approximately 10 hours for two sheds. The main barrier to the cleaning process was for the external contractor to find the farm, rather than anything associated with the cleaning activities themselves!

The cleaning contractors were not used to working with isolated sheep farms, most of their farm sector work being poultry based. The farmer trialling steam cleaning combined with disinfection is now considering buying or hiring a steam cleaner in the future instead of using external contractors.

For cold pressure washing, the cleaning of three cattle pens and three sheep sheds took 16 hours. The farmer that trialled this method will be using it in the future as he is hoping that it will help reduce general pathogen spread, including *Cryptosporidium,* in both his calves and lambs.

## 5.5 Feedback from farmers

Four of the participating farmers agreed to fill in the general feedback questionnaire about the project.

Three of them reported that *Cryptosporidium* was never diagnosed or suspected on their farm before the project. However, for two of those farms, *Cryptosporidium* was diagnosed in their livestock, during the project.

All farmers considered the project beneficial. They all reported that since the project started, they practiced higher levels of hygiene, specifically around shed cleaning.

Overall, all the farmers were very satisfied with the project and reported that the project increased their awareness and understanding of *Cryptosporidium*, and ways of reducing pathogen spread.

# 6 CONCLUSION AND LEARNINGS

Overall, the participating farmers were very satisfied with the project. It increased their awareness and knowledge about *Cryptosporidium* issues on farms, potential sources of infections as well as preventive measures that can be used to reduce both pathogen spread, incidence and production impacts.

The project helped the farmers gain a better understanding of pathogen spread via water courses, and the importance of increasing levels of biosecurity and hygiene in their indoor rearing sheds for youngstock. It also helped provide the knowledge and tools to tackle known or suspected issues on their farm. These include:

- Greater willingness to test for *Cryptosporidium* on farm and liaise with the veterinary profession on the topic.
- Improve hygiene practices during and in between lambing/calving, with an increased use of cleaning sheds down with pressure washing or steam cleaning combinations.
- The value of sampling water supplies for *Cryptosporidium* and then treat (for instance via installing a UV light) or limit access to certain water sources.

The project also further improved the liaison and relationship between the water company and farmers, working together on a topic which impacts common concerns, affecting both livestock and human health.

The project faced several challenges, compounded by the COVID19 pandemic, regarding farmer engagement and limitations on being able to sufficiently resource the sampling activities within the OG. Farmer engagement was a challenge initially, with preconceptions around the involvement of the water company impacting on farmer participation.

The sampling results provide an insight into the prevalence and range of strains of *Cryptosporidium* on livestock farms, and this project shows a greater level of appreciation of the presence of *Cryptosporidium* on-farm resulting in improved hygiene and biosecurity practices, and increased knowledge of pathogen spread on-farm.

The data gathered in this project has been added to Moredun's body of research on the topic and has been presented and cited at seminars -albeit anonymised- adding to the understanding of *Cryptosporidium* incidence and management on-farm in the UK.

*Cryptosporidium* will continue to present future challenges on these and other similar sheep farms, but this project has evidenced the benefits of farmers sharing information with each other on a difficult health issue, working with the veterinary sector, researchers and water companies.

## 6.1 Wider industry learning points

Project outcomes, which are of benefit to the wider industry include:

- Detail on the sources of *Cryptosporidium* infection on farms where it has been detected.
- Developing an understanding of the prevalence *of Cryptosporidium* in the farm environment.
- Adoption of procedures which will manage and/or prevent *Cryptosporidium* infection and sharing these approaches with the wider OG and the agricultural industry.
- Provision of recommendations on intervention measures that could be adopted to reduce the risk and prevalence of C*ryptosporidium* in surface and ground water, with specific reference to sheep and cattle systems in Wales.

In achieving these outcomes, the project contributes to knowledge that can influence the following environmental policy outcomes:

- Improved human health and welfare by sharing best practice for private drinking water supply protection.
- Improved animal health and welfare - particularly in youngstock.
- Improved resource management and efficient farm practice.
- Improved resilience in drinking water supply and treatment.

The literature review undertaken at the outset of the project demonstrated that the general pathways of *Cryptosporidium* transmission are poorly understood, as are specific sheep related factors associated with the pathogen. The project has provided new information on the prevalence and sources of *Cryptosporidium* in sheep enterprises.

The project complements ongoing Moredun research into this subject area. It provides learning which is being used to inform other *Cryptosporidium* in agriculture projects in Wales, working with Welsh Water.

## 6.2 Recommendations for future projects

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

- Good quality farmer and vet engagement is essential to the success of such projects.
- A partnership approach with wider organisations provides broader learning and access to expertise.
- Working with partners who have a perceived regulatory involvement can deter farmer participation if not handled carefully.
- Work with farmers who are genuinely curious about pathogens on-farm and want to understand more about ways of reducing disease spread -essential when investigating a pathogen, which is complex, resilient, and little is known about it in sheep systems.
- Good project design and organisation are essential to the success of this type of project.
- Sufficient administrative resource is required to support the project; for example, to contact and chase farmers to continue engagement with the project aims.
- Another key learning was the level of communication required to persuade farmers to take part, and the resource required to secure veterinarian and farmer time -both sectors have limited time available.
- The use of external veterinary specialists, who have credibility both within the farming and veterinarian communities helped ensure engagement.
- Working with a farmer facing animal health entity such as Moredun was critical in ensuring continuity, independent expertise, and provision of this in an accessible format for the farmer participants.

# 7 APPENDIX

## 7.1 Biosecurity review questions

Farm Name & Address:

Holding Number:

Vet & Veterinary Practice Details:

Type and number of livestock on farm

Sheep:

**A. Number of ewes**

Ewe Lambs kept for replacements

Rams

Store/Fat/breeding Lambs sold

**B. Open or closed flock:**

Are any of the following stock bought in:

Rams

Ewes/ewe lambs

Replacement newborn lambs

Fattening sheep

**C. Has your farm got multiple holdings the sheep move between:**

**D. Use of open hill:**

**E. Biosecurity with neighbouring farms on all holdings:**

Double fencing

Contact over gates

Contact over rivers/streams

**F. Use of summer or winter grass keep:**

If yes- is this ground grazed by other cattle or sheep from another holding at any time in the year?

**G. Quarantine procedures for sheep movements:**

Quarantine sheep bought in:

Quarantine sheep moved from open hill:

Quarantine sheep from other holdings/grass keep:

Where are sheep quarantined field/shed both:

Do you give any treatments associated with quarantine:

- Wormers/flukicides
- Treatment for lice/scab i.e., dipping.
- Footbathing
- Vaccinations

How long are bought ewe lambs/ewes quarantined before introduced to the main flock at lambing:

When introduced? Before lambing/during lambing/after lambing:

**H. Personal/visitors:**

Regular visitors on farm, including neighbours helping:

Do you have a disinfection policy for workers and visitors:

Do you have shared workers (contract lambers) with other farms:

Do you/or your workers change/disinfect waterproofs when handling different species cattle, sheep, birds etc:

Do you share equipment- trailers, muck spreaders etc:

Have you ever had illness among family or workers associated with stock on farm:

**I. Other stock kept on farm and approximate numbers, and how often new stock bought on:**

- Cattle
- Pigs
- Chickens/poultry
- Horses
- Cats and Dogs

Have there been health issues in cattle/pigs, etc Is there been cryptosporidium confirmed:

Does co-grazing occur between different species i.e., cattle and sheep:

If yes, does this occur within the lambing period or soon after turnout of lambs:

Are sheep sheds species specific, or do cattle/sheep use same sheds:

If use of the same shed, is the shed disinfected between use for cattle and sheep:

Do you share equipment between cattle/sheep:

Do you change/disinfect waterproofs when handling calves/lambs:

**J. What Wildlife seen on farm:**

- Wild birds-pheasants/grouse/game birds
- Rabbits
- Deer
- Rats
- Voles

**K. Feed storage:**

How is sheep feed kept- bins etc:

Can wildlife, cats/dogs access feed:

How is concentrate feed fed inside and out of shed:

**L. Watercourses/ drinking water sheep:**

Free flowing water access to grazing sheep-streams/rivers:

Mains water access in fields

Water access in sheds-mains/well water/free-flowing- piped from local streams/springs

If water is piped from free-flowing source/tank- is there wildlife access to this water source:

If so, do you use a filter/water purification before drinking water supplied to shed:

**M. How is muck/slurry stored on farm:**

How long is muck stored for before spreading:

How long a gap is left between muck spreading and grazing of grass by young stock less than 4 weeks of age:

**N. Lambing Protocols:**

Indoor lambing/Outdoor/Mixed:

Disinfection protocols in sheds pre-lambing/during lambing and post lambing:

What is your cleaning protocol:

When is shed cleaned-mucked out:

Disinfectant used/how is it applied/steam cleaning:

Bedding used in shed:

Is the shed cleaned out during lambing, if so, approximately how many weeks:

Protocols for disinfection- small and big pens during lambing:

Farm staff- disinfection of equipment/lamb feeders, milk machines:

Isolation facilities for scouring lambs:

Are gloves used/washing facilities in shed:

Are any medications used prophylactically-Spectam/Halocur in the sheep shed:

What other procedures are carried out before turnout i.e.: ring tails and castrating, scabivax, ear tagging:

Once lambs are turned out of the shed what fields are grazed within first 4 weeks:

Are lambs and ewes kept as batches on different fields:

Do the same fields get used for recently turned-out lambs for the whole of Lambing:

How are ewes fed once turned out of the shed:

**O. Outdoor lambing:**

Are the ewes paddock grazed or set stocked:

Are the ewes set stocked on fields heavily grazed before the start of lambing:

How long to newborn lambs and ewes remain on lambing fields before movement onto next grazing field:

Are the lambs handling at all during first 4 weeks of life before marking:

How are ewes fed in first 4 weeks post lambing:

**P. Flock Health issues:**

Has there been cryptosporidium diagnosed in flock previously:

Do you record illness/lamb losses during the lambing period.

What other diseases/illness is recorded in flock:

- Abortion
- Stillbirths
- Weak lambs
- Poor body condition score in ewes
- Scour in lambs
- Joint ill in lambs
- Sudden death/clostridial disease

Any other health issue in the flock: scab, fluke, border disease, MV, johns, etc

**Lambing Management Protocols:**

Is a protocol in place for all workers?

Small lambing pens and disinfection protocols

Colostrum management

Iodine on navels

How long in small/bigger pens before turnout

Any procedures carried out before turnout-scabivax, ringing, prophylactic treatments-antibiotics.

**On Farm Summary:**

Potential Sources of *Cryptosporidium*:

Control measures in place to limit spread of *Cryptosporidium* on farm:

## 7.2 Summary of the biosecurity responses for the seven participating farms

| | FARM I | FARM II | FARM III | FARM IV | FARM V | FARM VI | FARM VII |
|---|---|---|---|---|---|---|---|
| **No. of ewes** | 720 | 650 | 1,750 | 430 | 650 | 200 | 90 |
| **Bought in Sheep?** | Rams, occasionally ewes, 2 tiddling lambs | Only rams and 20-30 Welsh ewes (5%). | Rams bought in and some Welsh ewes. | 22 ewe lambs bought in and rams. | Rams and ewe lambs bought in. | Rams and some ewe lambs bought in | Only buy in rams |
| **Multiple holdings?** | Yes | No | Hoggs and hill ewes wintered in Pembroke. | No | No | No | Summer tack for ewes |
| **Use of open hill?** | Yes- common grazing | No | Hill is grazed along with other 4 neighbours. | Have common grazing. Ewes and lambs graze in May to weaning in August. Dry ewes then sent back. | No | No | No |
| **Biosecurity with neighbours** | 85% of boundary double fenced | Only join neighbours with two fields and possible contact over a stream. Otherwise, a road and forestry surround the farm. | Partly double fenced. Can contact neighbouring stock via walking gates and some areas of streams or rivers. | 90% of boundary double fenced with forestry on one side. Contact with one neighbour via stream. 5 grazers on hill. | Boundaries with neighbours double fenced. | No double fencing. No streams or rivers are shared with neighbours | All boundaries double fenced. Shared water courses |
| **Use of summer or winter keep?** | No | No | Cattle graze fields where hoggs and hill ewes are wintered. | No, only common grazing on hill. | Yes, but not grazed by other livestock at any time of the year. | Yes- summer grazing of ewes | Yes- summer tack for ewes |

| | FARM I | FARM II | FARM III | FARM IV | FARM V | FARM VI | FARM VII |
|---|---|---|---|---|---|---|---|
| **Sheep quarantine procedures** | 3-4 weeks separation from main flock for bought in sheep and sheep from open hill. | Quarantine bought in sheep for 21 days in field. Fluke, wormed and footbathed. | Rams quarantined for 14 days in fields. And wormed with Zolvix. Lincospectin used on all sheep's feet. | Bought in ewe lambs and rams separated for 3/4 months in field. Usually drenched for fluke + worm plus treated for scab pre-sale (OP). | Bought in sheep isolated in field for 21 days. Wormed and footbathed. | No procedures in place. | Rams- quarantine for 7 days, drenched and treat for lice. Ewes from tack- drench when returning. |
| **Personnel/ Visitor/other livestock details** | Shearing contractors mainly. Disinfect buckets etc between use of different species. | Only family help. Disinfect/change waterproofs to handle different species. | Nephew and young helper at lambing. Footwear disinfected in bucket. No disinfecting between handling different species. Machinery is shared. Family/worker had *Cryptosporidium* in past. | No visitors etc. No disinfecting between handling different species. No sharing of equipment. | Have part time workers- footwash supplied. Disinfect waterproofs before handling sheep after cattle. No sharing equipment. | No disinfectant policy. No regular visitors to farm. No shared equipment or workers. Will disinfect and change working clothes between working with cattle and sheep if a scouring problem is known. | Inspectors are asked to disinfect. No handling equipment shared. |
| **Other stock on farm** | 115 head of cattle. Cats and dogs. | Heifers bought in Spring. Also have horses, cats and dogs. | 117 cows, 61 store cattle, 2 bulls. 3 horses. 7 working dogs. | 15 cows with calves. 40 in total including stores. Five hens. Four dogs. | N/A | 30 suckling cows and calves. 3 dogs. | 50 cattle. 1 donkey. 2 ponies. 3 horses. Dogs and cats |
| **Health issues with other stock** | Scour in calves- 5-6 years ago. *Cryptosporidium* identified in scouring calf. | No *Cryptosporidium* confirmed previously. | No *Cryptosporidium* confirmed previously. | No *Cryptosporidium* confirmed previously. | No Cryptosporidium confirmed previously. | None identified. | BVD in cattle, being vaccinated. Rumen fluke in cattle. Have had Cryptosporidium in the cattle. |

| | FARM I | FARM II | FARM III | FARM IV | FARM V | FARM VI | FARM VII |
|---|---|---|---|---|---|---|---|
| **Fields co-grazed?** | Single species on own field. | yes- Cows and calves out with ewes and lambs. | Fields are co-grazed but not necessarily during lambing. | Sometimes co-grazed. No set time period left between cattle + sheep grazing. No co-grazing during lambing period. | N/A | Occasionally | No |
| **Are sheds species specific?** | Yes, but occasionally not at lambing-then disinfected | yes- Cows and sheep have separate housing. | Sheds are species specific, but equipment shared. | No, same sheds and equipment used for cattle and sheep but disinfected (Agristar) and mucked out between different species. | Sheep only | No | Yes |
| **Wildlife seen on farm** | Pheasants, Rabbits, Deer occasionally | Rabbits, Deer and no rats currently. | Pheasants, rabbits, one deer seen, rats. | Only rabbits on common grazing ground. | Pheasants/game birds, Rabbits, Voles. | Rabbits, pheasant and rats. | Pheasants, hares, foxes, badger. Rodents. Kites and Buzzards |
| **Feed storage** | Stored in bins. Concentrate always fed in troughs. | Sheep feed kept in bird proof bins. Animals fed in troughs. | Stored in bins or dumpy bags. Wildlife can access dumpy bags. Concentrate fed on silage or troughs for sheep. Feeders for cattle. | Stored in sealed bin in shed. No ad-lib feeding. Cattle fed in raised troughs. | Feed kept in bins and shipping container. Wildlife secure. Concentrate fed in troughs. | Stored in a wildlife secure container. Fed in troughs | Stored in a wildlife proof container |

| | FARM I | FARM II | FARM III | FARM IV | FARM V | FARM VI | FARM VII |
|---|---|---|---|---|---|---|---|
| **Watercourses/ drinking water** | 60-70% from Cwm Aran river. Spring well used for sheds with no *Cryptosporidium* found in the water. | One small stream starts on farm then flows to neighbours. Farm has borehole to supply fields, sheds and house. Wildlife could access water. Tested every 2 years for human consumption. | Free flowing water access shared with neighbouring farms. Mains water troughs in fields and sheds. | Most fields on the farm have access to a river. Only 2/3 fields have mains water. All sheds on mains. | Water tanks in most fields, some with free-flowing water access. Water in sheds from Well. No wildlife access. | Mains water supply for fields. Private water supply for sheds. | Water supplied from a mixture of open water courses or from the borehole. |
| **Muck storage and procedures** | Stored for minimum 3-9 months. No muck on grazing fields, otherwise left for 2 months before grazing. | FYM stored for 6 months on hard standing and spread in Autumn, no livestock access. | FYM stored on concrete next to shed. Sheep FYM stored 12 months, 3-4 months for cattle. Month gap between spreading and grazing. | Muck stored for 3 months before spreading. 8-month gap between spreading muck and grazing by young stock. | Muck stored alternatively on fields for 16 months, away from watercourses. 6-week gap between spreading and grazing. | Muck is spread straight onto field from shed. Not stored. Only a few days left between spreading and grazing. | Temporary field heaps. Cattle and sheep muck are stored separately. Do not spread until it has been rotting for over 12 months. |
| **Lambing protocols- indoor** | Fresh Wheat/Barley straw and lime daily and between each ewe in small pens. Shed pressure washed and disinfected. Sick pen for scouring lambs. Spray on navels. Facilities and equipment all disinfected and washed. | Sheds disinfected (Virkon) and mucked out in Autumn, overalls worn + PPE + foot dip. Straw bedding. Scouring lambs separated. Iodine on navels. Penned for 1-3 days. Spectam and Alamycin La used. | Feet disinfected. Lime sheds before housing. Hydrogen peroxide used now for disinfecting in watering can. Small pens limed between ewes. Disinfectant by the door. Straw used for bedding. Iodine on navels. Lambing equipment deep cleaned often. Hospital pen for | All sheds cleaned and disinfected pre lambing. Extra straw in small pens. Disinfectant sprayed to straw if E. coli is a problem. Barley/Wheat straw used as bedding. Lambing equipment cleaned with Agristar. No isolation facility. Spectam used for all twin lambs. | Fresh straw daily. Individual pens disinfected between use or re-sprayed. Shed mucked out within 2 months post lambing. Wheat straw used for bedding. Powdered disinfectant used. Lambing equipment disinfected. Iodine on lambs' navels. Isolation pen for | Indoor lambing. Sheds are cleaned and limed before housing with all small pens limed after every ewe. Small pen will be mucked out if it had a scouring lamb in it. The sheds are mucked out after housing for 6 weeks, before they start lambing and withing a few weeks of | Disinfect sheds with chemicals and lime the sheds before lambing. During lambing, top up the litter every 2 days. Lambed ewes are moved to small individual pens which are iodined and fresh bedding applied. Go outside straight |

| | FARM I | FARM II | FARM III | FARM IV | FARM V | FARM VI | FARM VII |
|---|---|---|---|---|---|---|---|
| | | | scouring lambs. 1 bottle Spectam used. | | scouring lambs. Spectam used on last 150 lambs. | lambing finishing. Milk bottles are washed after every feed. Gloves are used when in the lambing shed. No medication given at birth only iodine on navel. Ring castrated at turn out. | from individual pens. |
| **Procedures for turning out lambs** | All lambs tails ringed and scabivaxed. Ewes and lambs turned direct to fields and kept in small groups/batches. Ewes still fed up to 8 weeks post lambing. | Lambs' tails ringed and castrated. Moved to fields near buildings first, then further in groups of 10 ewes with twins, to larger groups of 30-40 pairs of twins. Ewes fed in troughs outside. | Crossbred lambs' tails ringed. Ewes and lambs kept as batches after turnout. Try to turnout to clean fields. | Lambs' tails ringed and castrated. Lambs turned out to same field from sheds and ewes fed. Then moved and kept in batches. Ewes fed for a minimum of 4 weeks after lambing. | Lambs' tails ringed and castrated. Turn out to close by fields but not always the same ones. Kept as batches on different fields. Ewes with multiple lambs still fed outside in troughs. Ewes and newborn lambs moved after 3 weeks. | Ring castrated at turnout. Turned out to fields closest to the yards/sheds and moved further afield as they get older. Kept in batches according to age. The same fields are used for recently turned-out lambs throughout lambing. Set stocking. Ewes are fed concentrates in troughs on ground. Period of supplementary feeding weather dependant. | Ring and tag lambs before turning out. Ewes are worm drenched at turnout and placed in a clean field. |

| | FARM I | FARM II | FARM III | FARM IV | FARM V | FARM VI | FARM VII |
|---|---|---|---|---|---|---|---|
| **Lambing protocols-outdoor** | N/A | Paddock graze ewes to lamb in fields surrounding house. Newborn lambs moved once a day. Ewes fed in troughs. | Ewes are not set stocked outside for lambing and moved from lambing fields a few days after lambing. Twins are all marked. Ewes fed concentrates in troughs. | Ewes set stocked in heavily grazed fields pre-lambing. Moved then after a few days. Lambs not handled until about 6 weeks old. | N/A | N/A | N/A |
| **Flock health issues** | *Cryptosporidium* detected in cattle previously but not in lambs. Had scours- *E. coli* previously in lambs. Had abortion issues before- Campylobacter? | Newborn lambs scouring in 2021 at 24 hours old. Footrot an issue. *E. coli* previously diagnosed in flock. Some joint ill issues occasionally. All illness/losses recorded during lambing. | *Cryptosporidium* previously diagnosed in lambs. Occasional lamb aborted- Inject for Enzo. Occasional stillbirth, weak lambs, joint ill or due to clostridial disease. Inject lambs with Bravoxin. Hill sheep dipped and dose for fluke. | N/A | E. coli confirmed in lambs previously but no Cryptosporidium. Lamb losses all recorded. Other conditions recorded are ewes in poor body condition, scour in lambs and sudden deaths/clostridial diseases. | No Cryptosporidium diagnosed. Occasional still birth, weak lambs, joint ill and sudden death/clostridial disease. Dose for fluke and worms and treat for scab with injectables. | Worm and fluke. Did have lice issues but is kept under control through treatment. |

| | FARM I | FARM II | FARM III | FARM IV | FARM V | FARM VI | FARM VII |
|---|---|---|---|---|---|---|---|
| **Summary** | Potential source of *Cryptosporidium* is a bought in calf. Procedures to control is to disinfect as often as possible. | Potential source of *Cryptosporidium* would be the stream. Measures to control are to test young lambs. | Potential source of cryptosporidium would be bought in calves in 2021. Control measures are disinfecting with Hydrogen Peroxide and dunking footwear in disinfectant. | No identification of potential *Cryptosporidium* source. No control measures currently to prevent *Cryptosporidium*. | E. coli confirmed in lambs 18 hrs old last year. Potential source of Cryptosporidium is Bore hole as not tested for house or stock. From streams or neighbouring farms. Control measures are checking for scours, using drink troughs. May fence off streams and good hygiene at lambing. | No identification of potential Cryptosporidium source. No control measures currently in place to prevent Cryptosporidium. Running the risk of buying it in. | Concerns that there were Cryptosporidium in the sheep flock. Were aware of Cryptosporidium in cattle already. |