1. **Roadmaps for the development of diagnostic tests and therapeutics for helminths**
2. **Roadmaps for the development of candidate vaccines and control strategies for liver fluke and nematodes**
3. **Roadmaps for the development of candidate vaccines, diagnostic tests and control strategies for FMD**
4. **Roadmap for research to underpin the development of control strategies for ASF**

SIRCAH Deliverable 3.4

30/09/2019

Interactive versions of the roadmaps in this report can be found at https://roadmap.star-idaz.net

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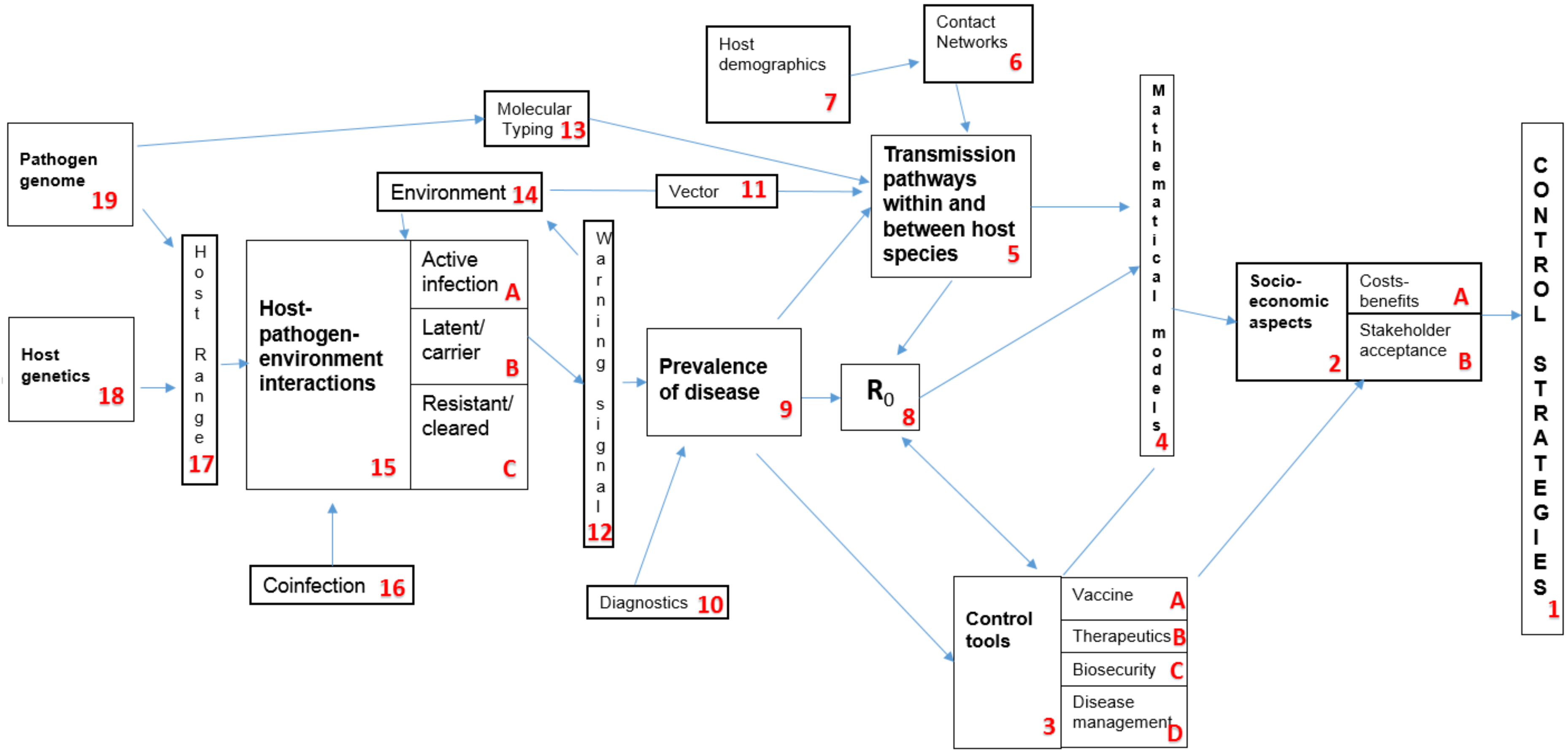
2aii) Roadmap for the development of control strategies for liver fluke



The roadmap for development of Trematode control strategies has been developed by the Livestock Helminth Research Alliance (LiHRA; June 2019) with major contributions of Grace Mulcahy, Diana Williams, Philip Skuce, Eric Morgan and Jozef Vercruysse

Liver fluke control strategies - Lead Summary 1

Title:	The sustainable control of *Fasciola hepatica*, minimising production loss while maintaining efficacy of therapeutics

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Sustainable control of liver fluke minimising production losses while delaying the development of resistance to therapeutics.
The major problem species is *Fasciola hepatica*. Other liver flukes, especially *Fasciola gigantica* and *Dicrocoelium dendriticum* cause problems in some regions. Trematodes in other organs, notably rumen fluke *Calicophoron* spp. and *Paramphistomum* spp., are also economically important. This summary focuses on *F. hepatica*.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Parasites can be controlled with anthelmintics but their frequent use results in the development of resistance.
The parasite modulates host responses preventing the development of protective immune responses.
The cost of control methods must be economically viable.

Infection and production loss persist on many farms in spite of control measures and more efficient use of supportive control approaches such as grazing management is needed but difficult to achieve in practice, especially as epidemiological patterns are altered by climate and management change.
Fasciola hepatica is zoonotic and causes significant human disease in some localities.

Solution Routes

What approaches could/should be taken to address the research question?

The validation of treatment regimens that minimise the development of anthelmintic resistance.
The development of alternative control methods such as vaccination, pasture/grazing management.

Dependencies

What else needs to be done before we can solve this need?

Cost benefit analysis of the various treatment options.
Knowledge of stakeholder acceptability.
Development of vaccines.
Development of new therapeutics.
Development of pasture management strategies that minimise disease, including appreciation of the impacts of climate and management change on disease risk.
Knowledge of the basis and extent of variation in susceptibility between host species and breeds.

State of the Art

Existing knowledge including successes and failures

Basic life cycle and epidemiology is worked out but important details missing, e.g. on intermediate host range and dynamics, immune responses, genetics of resistance, and alternative control methods. Native antigen vaccines have shown promising efficacy in trials but recombinant vaccines have not yet been shown to induce protective responses.

Projects

What activities are planned or underway?

Ongoing research projects across the world, focusing on epidemiology and control, drug action, vaccine development, intermediate host range and capacity, parasite genetics.

Liver fluke control strategies - Lead Summary 2A (Socio-economic aspects-cost/benefits)

Title:	Economic evaluation of current and future control strategies against liver fluke

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What are the production and economic consequences of anthelmintic resistance (AR) in liver fluke?
How to develop tools to quantify the economic impact of liver fluke infections at global, national, regional and farm level to support decision-making by governments, animal health organisations and farmers?

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Current systems use average production estimates and thus lack farm-specificity, even though fluke impacts are highly variable between farms and even within farm territories. Current approaches to economic assessment are also based on partial budgeting and do not reflect the effect on the whole-farm economic performance.

Solution Routes

What approaches could/should be taken to address the research question?

Additional studies to determine the production impact of liver fluke infections in various geographical settings, with inclusion of impacts on fertility.
Use development of various economic modelling approaches and adapt them to helminth infections.

Dependencies

What else needs to be done before we can solve this need?

A closer collaboration between the model-makers and model-users and the stimulation to develop concrete business cases may be the critical success-factor for these systems to become self-sustainable in the near future.
Linking economic models to real data collected on farm, rather than economic models that estimate impact based on previous knowledge on productivity impact only.

State of the Art

Existing knowledge including successes and failures

Whereas an increasing amount of data are being generated for the direct production impacts of liver fluke infections, more emphasis should now be given to the production and economic impacts of AR. Over the last decade, good progress has been made in assessing the production economic impacts of helminths in ruminants, and these have extensively been reviewed in sheep and in cattle. A remaining gap is to establish the impact on fertility parameters using randomized intervention field studies. However, the major challenge is to develop tools that are able to quantify the economic impact of liver fluke at national, regional and farm level to support decision-making by various stakeholders and that can be used as management tools.

Projects

What activities are planned or underway?

Partial budget analysis at a national level has been completed for the UK and will be published shortly. Farm level economic models, incorporating real on farm data have also been produced (papers submitted for publication).

Liver fluke control strategies - Lead Summary 2B (User acceptance)

Title:	Sociologic evaluation to understand user needs and acceptance of control strategies against liver fluke

Research Question
What are we trying to achieve and why? What is the problem we are trying to solve?
How can we improve the development and uptake of best practice management of liver fluke control?

Challenge(s)
What are the scientific and technological challenges (knowledge gaps needing to be addressed)?
Farmers are often not aware of the cost of disease. Recommendations for control have changed over the recent past, so to whom should farmers listen? The benefits of integrating diagnostics and treatment have not been widely recognised or applied: few farmers use any diagnosis. Farmers are frequently confused by the range of products available and the active ingredients. Prescribing advice needs to be improved and knowledge exchange programmes are required to achieve this. The targets of these programmes vary between countries according to routes of anthelmintic sale.

Solution Routes
What approaches could/should be taken to address the research question?
Establishing the motivational factors behind the decisions of farmers.
Development and extension of decision support tools that can act alone, but can also be integrated into general farm or pasture management software, e.g. farm-level risk mapping. Development of liver fluke control decision support systems for cattle, sheep and goats. Demonstration trials showing the feasibility and beneficial outcomes of best-practice approaches.

Dependencies
What else needs to be done before we can solve this need?
Long-term relationships with farming communities, allowing deep study of socio-psychological elements of decision-making. Cost-benefit analysis demonstrating the cost of disease and the economics of various treatment options.

State of the Art
Existing knowledge including successes and failures
Surveys exist of farmer knowledge, attitudes and practices in relation to liver fluke control, but more work needed to understand motivations in holistic socio-economic contexts.

Projects
What activities are planned or underway?
Socio-economic studies underway in UK; KAP studies delving into farmer behaviours on choice of flukicidal products.

Liver fluke control strategies - Lead Summary 3A (Control tools - vaccine) N.B. separate roadmap on liver fluke vaccine development

Title:	Requirements and use strategy for liver fluke vaccines

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*
Main problem is to achieve effective protection from a vaccine, consistent between individuals, and suitable for field use. Scalability for commercial use requires either recombinant vaccines or a system for producing effective native antigen vaccines at scale; both routes are challenging. Deciding what level of protection is useful in the field depends on further questions: How long should a vaccine protect livestock and what levels of protection would be sufficient to prevent disease and production losses? What would be the best way of administering the vaccine to reduce production losses, in combination with other control approaches including chemoprophylaxis and grazing management?

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
Why can protection be elicited using native antigen and not, so far, recombinant antigens? How do immune evasion and modulation strategies by the fluke circumvent vaccine efficacy? The levels of efficacy required will vary across livestock species and between regions, depending on climatic context and local farm management practices. Vaccine efficacy requirements may also be different in calves or lambs that co-graze with their dams.

Solution Routes *What approaches could/should be taken to address the research question?*
For logistical, financial and animal welfare reasons, it will be practically impossible to test all possible scenarios by vaccine trials in the field. Evaluating vaccine efficacy requirements through mathematical modelling is a valuable tool to help define useful levels of protection and to model integrated use of vaccines with other parasite control measures. Ultimately, producing a vaccine effective enough to make an epidemiological impact is the main solution route.

Dependencies *What else needs to be done before we can solve this need?*

At present, regulatory authorities are not familiar with registration of helminth vaccines. As it is unlikely that any vaccine will obtain efficacy levels that are comparable with those of modern anthelmintics (or vaccines against viruses and bacteria), regulatory authorities will need to be informed about thresholds for duration and level of protection that are sufficient to reduce infection to a level that does not interfere with animal welfare and productivity.

State of the Art

Existing knowledge including successes and failures

One modelling study study has been published attempting to set epidemiologically useful thresholds for protection in the field, but this is theoretical only and acknowledged unexplored variation across climates and management systems.

Projects

What activities are planned or underway?

Vaccine trials continue using various refinements to attempt to increase protection level.
It is assumed that natural immunity to fluke infections is limited but reasons for this and prospects of surmounting this through vaccination are subject to ongoing research.

Liver fluke control strategies – Lead Summary 3B (Control tools - therapeutics)

Title:	Sustainable use of flukicides

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What is the prevalence of anthelmintic resistance in liver fluke populations to different actives, and how is this distributed?
Can the implementation of best-practice management reverse the anthelmintic resistance status on a farm?
Are refugia-based strategies for liver fluke control appropriate biologically and in terms of farmer willingness? How large must the *refugia* be and how long should it persist in order to minimize selection for AR? Is there a fitness cost to refugia? What is the proportion of animals that should be left untreated? How can we improve the timing of treatment?
To what extent can alternative control methods, including grazing / pasture management, reduce the need for treatment and hence alleviate selection for resistance?

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

To date, there are >20 peer-reviewed reports of TCBZ-R on sheep farms within Europe, plus a number of anecdotal reports of resistance, which raises the question how prevalent is TCBZ-R in Europe. Occurrence outside Europe is less well documented. It is not clear if reports of drug failure are effectively recorded through veterinary medicine surveillance schemes in different countries and if so, if these data are available in the public domain. This information is needed to provide evidence for the widely held belief that resistance is widespread across northern Europe and to give a clear picture of the TCBZ-R status at an individual farm level, to ensure the most effective control measures are employed.
Better tests are needed to detect resistance. FECRT to detect resistance to TCBZ is validated for use in sheep but not cattle.
Risk that farmers switch to other actives, leading to heavy use of e.g. closantel with risk of resistance emerging. Need for surveillance of resistance to closantel as well as TCBZ.

Solution Routes

What approaches could/should be taken to address the research question?

Quick and reliable test to detect AR in the field. Approaches to refine FECRT (e.g. faecal pooling) and use of other diagnostic tools (e.g. copro-antigen reduction) should be further investigated.
Field surveillance data on progression of AR and the impact of alternative strategies to slow it.

Dependencies

What else needs to be done before we can solve this need?

Development of new therapeutics, especially to cover immature flukes and acute disease.
Development of molecular diagnostics for AR in liver fluke.

Field studies of the integration of therapeutics in disease management strategies.

State of the Art

Existing knowledge including successes and failures

Recently, it was found that if the use of multiple active anthelmintics is combined with “best practice parasite management,” based on avoiding overuse of anthelmintics, minimizing nematode challenge to susceptible animals, and maintaining a nematode population in refugia, resistant populations may even be reversed towards susceptibility. This concept, and the use of refugia-based strategies in general, remain unexplored for liver fluke. However, refugia-based and community replacement approaches trialled for nematodes might not be appropriate for liver fluke because of the high pathogenicity of the parasite and its capacity for rapid population increase through asexual reproduction in the snail host. This requires further research before recommendations are prematurely made. The use of multi-active or combination anthelmintic approaches, which are increasingly advocated for nematodes, also have not been assessed for liver fluke.

Projects

What activities are planned or underway?

A number of nationally-funded projects on advancing fluke diagnostics and especially markers for early infection and extent of infection, including both refined markers (e.g. copro-antigen, copro-DNA) and simple clinical guides (e.g. FAMACHA/five-point check). Studies to integrate new diagnostics into targeted treatment approaches.

Liver fluke control strategies – Lead Summary 3C (Control tools - biosecurity)

Title:	Preventing the introduction of AR parasites

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*
Prevent the introduction of *F. hepatica* and/or resistant parasites onto farms where either are not already present.

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
The degree to which AR develops on each farm versus its introduction with purchased animals. How effective quarantine measures can be against liver fluke and AR. Farmers' willingness to adopt quarantine measures, and how these should be designed: length of time of isolation, identification of appropriate pasture risk for turnout.

Solution Routes *What approaches could/should be taken to address the research question?*
Field and modelling studies to document and increase efficacy of quarantine procedures on livestock imported onto farms. Population genetic approaches to evaluate relative importance of AR spread between farms versus on-farm selection, following recent approaches for nematodes.

Dependencies *What else needs to be done before we can solve this need?*
Pen-side diagnosis of fluke infection and AR. Better understanding of the genetics of resistance in liver fluke.

State of the Art *Existing knowledge including successes and failures*
Solid field evidence for the effectiveness of different strategies to limit AR spread and development is lacking. For trematodes, complex genetics (ploidy) and involvement of intermediate host in which clonal expansion occurs make population genetics even more difficult than in nematodes. Cost of quarantining and testing animals being brought in to the farm might limit practical application.

Projects *What activities are planned or underway?*
Some research on wildlife reservoirs of fluke and how they might affect quarantine; surveys of farmer practices in relation to quarantine.

Liver fluke control strategies – Lead Summary 3D (Disease management)

Title:	Integrated control through pasture management and other control options

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

To minimise the level of infection and production losses through improved management, integrating all of the various control methods.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

There is a lack of evidence on how environmental / pasture / grazing and other tools can be integrated and used to decrease reliance on chemical control and alleviate development of AR.
Empirically-derived understanding might be limited under different conditions, e.g. as climate and management change.
Possible external sources of fluke eggs, e.g. from deer, lagomorphs and nutria, might need to be taken into account.

Solution Routes

What approaches could/should be taken to address the research question?

Field studies that use and attempt to optimise integrated control, and quantify the benefits, are needed.
Modelling studies can help to generalise the conclusions and apply them to systems where field data are limited, as well as to narrow hypotheses and design efficient studies.

Dependencies

What else needs to be done before we can solve this need?

Knowing prevalence of infection and AR, hence the need to change approach.
Better understanding of environmental drivers of infection is needed to effectively apply integrated control.
Studies rely on methods to detect infection and these could be improved, e.g. quantification of metacercariae on pasture; detection of eDNA for fluke and snails.
Solutions limited by the practicality of fencing off or draining waterlogged areas.

State of the Art

Existing knowledge including successes and failures

Empirical knowledge of the topographical and other environmental determinants of liver fluke risk within farms is sound but avoidance strategies are still not widely applied.
Predictive understanding is limited to correlates of high infection risk (topography, climate).

Projects

What activities are planned or underway?

eg eDNA work in UK, also environmental metacercarial challenge studies

Liver fluke control strategies – Lead Summary 4 (Mathematical models)

Title:	Mathematical models of liver fluke for prediction and control

Research Question
What are we trying to achieve and why? What is the problem we are trying to solve?

Can we develop mechanistic mathematical models to improve on current forecasting systems, which are only applicable to certain regions, and to evaluate novel control strategies?

Challenge(s)
What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Current forecasting systems are empirical and as such cannot be fairly extrapolated to other regions or to future changes because they do not explicitly capture the dependence of the life cycle of *F. hepatica* on key environmental factors.
Mechanistic models might be more robust under different and changing conditions, but require detailed knowledge of the life cycle and its dependencies.

Solution Routes
What approaches could/should be taken to address the research question?

Better insights into the effects of environmental conditions on the survival of eggs, metacercaricae and intermediate host snails on pasture in different geographical settings.
Knowledge of the population dynamics of snail intermediate hosts (IH), and effects of climate and pasture conditions.
Mathematical and computational model frameworks that are able to address key drivers of fluke epidemiology at a range of scales, for regional forecasting, and within-farm decision support.

Dependencies
What else needs to be done before we can solve this need?

Field data on prevalence of infection under a wide range of conditions, with which to validate new modelling approaches.
Tools to more accurately measure metacercariae density on herbage, to validate and refine models at farm level.

State of the Art
Existing knowledge including successes and failures

Empirical forecasting tools, notably the Ollerenshaw or Mt model, have proven useful in identifying higher risk years and are disseminated to farmers, e.g. in the UK. Environmental correlates of high risk are also well described at regional, farm and individual field scale. There have been some attempts to build mechanistic transmission models but validation has been limited and focused on the same areas.
Models of within-host processes have been produced, and these would be enhanced by greater understanding of host responses.

Projects

What activities are planned or underway?	A number of new modelling approaches including adaptation of high-resolution hydrologic models to predict fluke risk at within-farm field level.

Liver fluke control strategies – Lead Summary 5 (Transmission pathways within and between host species)

Title:	Role of the intermediate hosts in liver fluke epidemiology

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

How do intermediate host snail biology and infection drive *Fasciola hepatica* epidemiology in definitive hosts?

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Understanding of snail population dynamics and factors that affect transmission are implicit rather than explicit.
Biological influences on snail populations, e.g. through predation and parasitism, largely unknown.
Lack of understanding of the snail immune response to *F. hepatica*.
Variation between snail species and strains in receptivity to *F. hepatica*, and the importance of within-host processes including innate immunity and competition from other trematodes in determining the fate of incoming miracidia, largely unknown.

Solution Routes

What approaches could/should be taken to address the research question?

Using remote sensing methods, particularly soil moisture data from the new generation of Sentinel satellite systems together with other technologies, such as detection of environmental DNA, to identify suitable snail habitat on farms will improve our ability to predict when and where metacercariae may appear on pasture. The timing of high-risk periods will vary from region to region.
A practical and accurate tool to measure cercarial levels on pasture and their viability would be a big step forward.
Basic biological studies in a farming context to determine how far snails roam, the distance cercariae travel before encysting and what factors trigger cercarial shedding

Dependencies

What else needs to be done before we can solve this need?

Further validation of PCRs to detect *F. hepatica* infection. It is important to ensure that putative trematode-specific PCR primers do not amplify snail DNA. A number of other trematode species, including those of birds and amphibians, have been isolated from *G. truncatula* including *Calicophoron daubneyi*, *Haplometra cylindracea*, *Notocotylus* spp., *Plagiorchis* spp. Some of these trematodes have little or no published DNA sequence available, which makes it difficult to ensure PCRs are *F. hepatica* specific. Understanding the role of these other trematode infections on the dynamics of *F. hepatica* in snails as well as parasite detection should be improved.

Lack of methods to identify when metacercariae appear on pasture and to quantify risk.

State of the Art

Existing knowledge including successes and failures

The presence of the snail intermediate host is essential to the transmission of *F. hepatica,* and knowledge of the interaction between snail and parasite is important when considering what drives parasite transmission. It is also important to understand how events in the snail influence genetic diversity of parasites in the mammalian host. To fully understand the epidemiology of *Fasciola* spp., better knowledge of snail habitats, species of snails acting as intermediate hosts, and prevalence of *F. hepatica* infection within the snail are required.

For many years, the role of the intermediate host in the *Fasciola* spp. life cycle has been relatively neglected, but modern molecular and genomic tools are becoming available to study events in the snail, and we can start to address how these impact on transmission and the spread of virulence and anthelmintic resistance genes within fluke populations.

Projects

What activities are planned or underway?

Studies to improve understanding of role of non-classic lymnaeid species at margins of range, quantification of traits such as cercarial output, and population dynamics of snail hosts.

Liver fluke control strategies – Lead Summary 6 (Contact networks)

Title:	Role of livestock movements and wildlife reservoirs

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What is the role of livestock movements and wildlife reservoirs in the spread of liver fluke infection in general and of resistant genotypes in particular?

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Effective contact between hosts is a function of grazing shared pasture, with transmission depending on grazing high-risk pasture subsequent to a contamination event and subsequent development and persistence of metacercariae. Contact networks in this case become pasture grazing maps allied to topographical and climatic influences on the life cycle. These are complex to construct, farm-specific, and difficult to validate.

Solution Routes

What approaches could/should be taken to address the research question?

Need for empirical and theoretical studies at a range of scales to determine how contact through shared grazing drives transmission and the implications for the spread and development of resistance.

Dependencies

What else needs to be done before we can solve this need?

Need more molecular markers of infection and AR.
Mechanistic rather than only empirical prediction tools.
More precise ways to capture and record livestock movements within farms.
Quantification of the role of wildlife in fluke transmission within and between farms.

State of the Art

Existing knowledge including successes and failures

General understanding of the fluke life cycle permits 'rule-of-thumb' estimation of duration and level pasture infectivity. This is applied in practice but could be refined by taking a contact network approach, with pastures as nodes.

Projects

What activities are planned or underway?

Some surveys continue on wildlife host species and their contribution to farmland contamination.

Liver fluke control strategies – Lead Summary 7 (Host demographics)

Title:	Animal characteristics

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*
The impact of the age structure of a herd/flock on fluke contamination levels and transmission

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
Differences in the response of cattle and sheep. The basis of age-resistance in cattle, and whether this represents a real difference in susceptibility that can be used to aid control.

Solution Routes *What approaches could/should be taken to address the research question?*
Field monitoring of infection levels and dynamics in different age cohorts across the whole farm production cycle. Basic research on the immunological differences between different animal species and ages.

Dependencies

What else needs to be done before we can solve this need?
Immunological tools for ruminants are some way behind those for other animal models.

State of the Art *Existing knowledge including successes and failures*
Repeated infection appears to make the liver more refractory to infection, but this has not been adequately tested or its basis fully explored. Sheep and cattle differ in immune response but again the basis is not known, even to the extent of being physiological or immunological in nature. Level of difference between breeds in susceptibility and response is not known.

Projects *What activities are planned or underway?*
NK

Liver fluke control strategies – Lead Summary 8 (R_0)

Title:	Parasite population amplification and its contribution to disease risk

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What are the important factors governing the amplification of fluke populations and how does this assist propagation of infection?
Can onward propagation be reduced to below the threshold for population persistence and what is the most efficient way to achieve this?

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Level of asexual reproduction within snail hosts appears to vary widely but the role of different factors (e.g. snail genotype and age, competition within snails, microclimatic conditions, parasite strain) has not been elucidated.
Estimation of R0 for fluke supposes construction and calibration of a mechanistic transmission model, which does not yet exist.

Solution Routes

What approaches could/should be taken to address the research question?

Well-targeted experiments to calibrate R0 estimation.
Exploration of whether an R0-type model can replicate or improve on the performance of empirical risk models.

Dependencies

What else needs to be done before we can solve this need?

Similar areas of uncertainty to mathematical models (see 4).

State of the Art

Existing knowledge including successes and failures

This would be a new approach to fluke epidemiology and could complement other methods.
Mechanistic, stochastic and R0 models are under development and preliminary models have been published.

Projects

What activities are planned or underway?

Several new modelling approaches under way; data for validation unclear.

Liver fluke control strategies – Lead Summary 9 (Disease prevalence)

Title:	Liver fluke prevalence

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Can we set up a monitoring system to detect changes over time and act as decision support tool on European level?
Need to develop standardised surveillance systems.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Lack of harmonisation of diagnostic systems.
Needs country or even continental-based approach.
More effective use of abattoir data and systems to trace animals' movement history to identify source farms and where infection occurred.
Set up network of sentinel farm also for other infections.
Need to assess minimal required sample size.
Impact is actually related to level of infection and not just prevalence so need to determine the extent to which prevalence, or prevalence-above-threshold, can be a useful synoptic measure to characterise baseline infection and detect changes.

Solution Routes

What approaches could/should be taken to address the research question?

Use of existing sample collection schemes based on bulk tank milk monitoring programmes and veterinary and hunting networks for the collection of faecal samples from non-dairy livestock and wildlife. Collection and collation of abattoir data.

Dependencies

What else needs to be done before we can solve this need?

Harmonize or integrate diagnostic and data collection systems across various countries and regions.
Assess cost-benefits of such an approach and develop how this can be effectively communicated.

State of the Art

Existing knowledge including successes and failures

Complementary to predictive systems, it is important to set up surveillance systems that monitor infection status at farm level on a regular basis. Such systems can capture unexpected deviations from mathematical model predictions and indicate whether farmer management is able to cope with altered disease risk or not. Recently, it was shown that monitoring *F. hepatica*-specific antibody levels in bulk tank milk from a randomized sample of dairy farms allowed detection of both interannual (weather-driven) changes as well as longer-term trends in *F. hepatica* exposure. Moreover longitudinal monitoring approaches have also been shown to be an effective decision support tool.

Projects

What activities are planned or underway?

Liver fluke control strategies – Lead Summary 10 (Diagnostics) N.B. Separate roadmap for diagnostic test development

Title:	Liver fluke diagnostics

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

More accurate, convenient and affordable diagnostic tests are needed to support surveillance and to enable appropriate intervention at farm level.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Tests are available but all have limitations. Especially important is to determine the time of first infection in autumn in order to drive timely intervention, and tests for anthelmintic resistance, which are currently limited by low sensitivity of faecal egg counts and limited availability of genetic markers. Specific detection of immature fluke infections is needed but currently not available.
There are some candidate tests that showed promising results in individual laboratories, but these would have to be standardised for widespread use and clear interpretation.
Requirement of penside/point of care tests for immediate estimate of infection (e.g. for quarantine treatments/choice of treatment of individual animals or groups of animals).

Solution Routes

What approaches could/should be taken to address the research question?

Tracked longitudinal experimental and natural infections with candidate diagnostic indicators of early infection.
Correlation of tests for level of infection with productivity to determine thresholds for intervention.
Molecular genetic approaches to find markers of resistance.
More sensitive and convenient ways of extracting and enumerating eggs in faeces to support quantification of infection and drug efficacy.
Ring-tests to confirm consistency of results between laboratories as a precursor for widespread use and commercial adoption.

Dependencies

What else needs to be done before we can solve this need?

Seeking tests for resistance depends on access to fluke populations of known drug susceptibility.
Tests for early infection require known timing of infection, hence experimental animal studies.
Tests intended for surveillance and on-farm decision support must be cheap and convenient enough for easy application.

State of the Art

Existing knowledge including successes and failures
Promising advances include copro-antigen and copro-DNA tests for early fluke infection, bulk and individual milk immunodiagnostics for fluke-specific antibody levels, and pooled faecal egg count tests to estimate drug efficacy. Monitoring tegumental changes in juvenile flukes recovered from slaughtered animals and incubated in triclabendazole can be used to evaluate resistance but are a research tool only. As in nematodes, in vitro tests for resistance either by bioassay or using molecular indicators remain held back by limited knowledge of the mechanism and genetic basis of resistance to different drug classes. This impedes development of resistance tests that can be reliably used in the field, and be convenient and affordable enough to be taken up by farmers and advisors.

Projects *What activities are planned or underway?*
Ongoing work in this area in several laboratories.

Liver fluke control strategies – Lead Summary 11 (Vector Biology)

Title:	Biology of the intermediate host snails

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The role of the intermediate host in maintaining and transmitting infection.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Do parasites differ in their virulence for the intermediate host?
The development of biological and other alternative control methods for the snail.
Host range of the parasite and ability to use alternative snail species in different parts of the world and different environments to expand endemic areas.
Competition within and between trematode species for resources within the snail and outcomes for fluke transmission.
Knowledge of snail-parasite interactions is essential to predict how snails will adapt to infection pressure and altered management.
Impact of climate change on snail biology/adaptation to different environments

Solution Routes

What approaches could/should be taken to address the research question?

Better understanding of basic snail biology.
Resumption of work on basic population dynamics of snails.
Integration of studies on fluke intermediate hosts with ecological theory and practice.
Development and application of novel molecular tools to better understand heterogeneity in snail-parasite interactions and its implications.
Experimental approaches to snail defence and parasite strategies for overcoming them.

Dependencies

What else needs to be done before we can solve this need?

Knowledge of the impact of environmental factors on the vector.
Methods for determining infection levels in snails.
Improved knowledge of snail-*Fasciola* interaction.

State of the Art

Existing knowledge including successes and failures

After basic work on transmission processes, much knowledge of the role of the intermediate host in fluke transmission relies on correlation of environmental factors with disease challenge in livestock, with snail populations implicated as the causative link.

Quantifying the processes at play is essential to upgrade predictive epidemiological understanding. Understanding these processes in more detail might also allow more effective use of environmental and alternative control approaches, including biological control. The effects of diversity in snail hosts on transmission potential has been explored but mainly at species level and not finer scales (between and within populations of the same snail species).

Projects *What activities are planned or underway?*

Liver fluke control strategies – Lead Summary 12 (Warning signal)

Title:	Early warning systems for liver fluke

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

How can we use environmental and other data to predict *F. hepatica* risk at various geographical levels?
Prediction of disease risk in space and time would allow farmers to react appropriately and help develop strategies by policymakers and industry.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Empirical approaches to prediction will always be limited by the scope of the data used to calibrate them, so involve substantial and ongoing work to enable expansion to other areas and to future conditions.
New data streams from remote sensing greatly enhance availability of environmental information but this must be matched by management and parasitological data to transform hazard prediction into risk prediction.

Solution Routes

What approaches could/should be taken to address the research question?

Recent studies have explored the use of very high-resolution satellite and drone imagery to map small water bodies and the intermediate host snails on pasture, but further research is required to make this approach operational and to develop sustainable business cases. Enhanced capability to monitor water bodies at high spatial resolution could be provided by recent sentinel European Space Agency (ESA) satellites, which carry a range of technologies, such as radar and multi-spectral imaging instruments for land, ocean and atmospheric observation (https://sentinel.esa.int).

Dependencies

What else needs to be done before we can solve this need?

Correlations between remotely sensed data and environmental drivers of transmission (e.g. water bodies versus soil saturation) must be demonstrated.
Systems for automated processing of streamed data on important environmental, climatic, management and parasitological factors, conversion to useful risk predictions, and delivery to end users in a timely and usable way.

State of the Art

Existing knowledge including successes and failures

Despite the important progress in this area, the spatial distribution of *F. hepatica* in southern, central and eastern

Europe, remains poorly described. Furthermore, additional research is required to improve the spatial resolution of *F. hepatica* risk maps from broad administrative or farm level to pasture level so that risk maps can support the implementation of specific management advices on drainage, grazing strategies and targeted (selective) treatments.

Projects *What activities are planned or underway?*

Liver fluke control strategies – Lead Summary 13 (Molecular typing)

Title:	Molecular epidemiology

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

What is the importance of different host species including wildlife reservoirs, management and animal movement on gene flow between populations?
How do different parasite genotypes vary in virulence and other biological traits, including response to host defences and therapeutics, performance in intermediate hosts, etc.?
Do susceptible and resistant parasite genotypes differ in biological fitness?

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

In contrast to their application in viral and bacterial infections, population genetic studies are an underexploited approach to unravel host-parasite co-evolution and there are no agreed methods and limited neutral genetic markers to conduct such studies.

Solution Routes

What approaches could/should be taken to address the research question?

Developing the methods for population genetic structure studies for *F. hepatica* and applying them to isolates collected from across the world. Including isolates from wildlife and combining with established livestock movement databases and standardized questionnaires on farm management.

Dependencies

What else needs to be done before we can solve this need?

Deeper knowledge of fluke genetics, especially for genes relevant to resistance.
Improved genetic map, better annotated genome resources, better understanding of heterogeneity and redundancy within key gene families.
Population and distribution data for relevant wildlife species and ways of measuring movement within and between farms and its implications for gene flow.

State of the Art

Existing knowledge including successes and failures

The population genetic structure of a parasite species has important implications for evolutionary processes such as adaptation to host defences and the development of AR. We still know surprisingly little about the population genetic structure of most species of parasitic helminths, in a livestock context. There is also widespread livestock movement at both national and international scale. Movement is normally undertaken with little or no monitoring or effective quarantine measures against helminth parasites. Wildlife, such as rabbits, hares, deer and others, may act as reservoirs of certain helminth infections for livestock. On the one hand, this may contribute to the parasite population *in refugia* but, on the other act to disseminate parasites and resistant genotypes. Population genetic studies represent an as yet under-exploited approach to unravelling the scale and drivers of parasite movements between farms.

Projects *What activities are planned or underway?*
Ongoing work in the UK is generating neutral genetic markers for use in population studies, and deeper understanding of parasite genetics and associated biological traits.

Liver fluke control strategies – Lead Summary 14 (Environment)

Title:	Environmental influences on liver fluke epidemiology

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The impact of climatic and land use changes on the snail vector and transmission of infection.
Role of temperature and moisture on egg development
Survival of the snail and various parasite stages in the environment.
How are parasites in *refugia* influenced under different environmental and management conditions?

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Described in sections 1, 4, 11, 12.
Environmental correlates are well described but only for certain regions, e.g. in the UK, Ireland, and Belgium. Need to extend studies beyond these areas and transform understanding into predictive approaches that can be fairly applied under new and future conditions.
Use of environmental management to support fluke control is widely advocated but limited by practical obstacles (e.g. incompatibility between economic factors / subsidy structures and alternative land uses) and other more powerful drivers of decisions at government and farm levels (e.g. flood control, agri-environment schemes). A major challenge is to conduct theoretical and field-based investigations into how integration of topographical, environmental and management factors can effectively reduce impacts of liver fluke and reduce reliance on failing chemical control.

Solution Routes

What approaches could/should be taken to address the research question?

Field studies are large, long, complicated and expensive and require appropriate levels of commitment from funders and stakeholders.
Theoretical approaches are not a substitute for field data but could help to identify clearly testable hypotheses and select the most effective study designs.
The potentially broad influence of interacting factors means that it would be very difficult to control for all the variables in such studies. Taking advantage of interventions driven for reasons other than fluke control, e.g. change in land management as a result of altered policy, might provide routes to test interventions against fluke.

Dependencies

What else needs to be done before we can solve this need?

Testing alternative strategies would require better tools for quantifying metacercariae load on pastures.

State of the Art

Existing knowledge including successes and failures

Existing risk predictions are based almost entirely on climatic drivers of infection (temperature and precipitation) and other environmental factors are neglected. Several have been shown to relate to infection levels (e.g. topography, soil type), but only in statistical and broader spatial models. There is a need to advance beyond this level of understanding such that changes in environmental drivers of infection can be used effectively to attenuate fluke transmission, or negative impact of environmental changes on disease risk anticipated and actions taken to protect animal health.

Projects

What activities are planned or underway?

Liver fluke control strategies – Lead Summary 15A (HPE interactions – active infection)

Title:	How does immunity develop against F. hepatica?

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Can we improve the quantitative understanding of acquired immunity against *F. hepatica* in sheep and cattle in order to understand demographic influences on infection, support vaccine development, and incorporate this in mathematical models of parasite epidemiology?

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Understanding of immune responses to fluke infection is rudimentary and derived mainly from vaccine trials. There is a need to renew focus on the fundamental immune processes in actively infected animals, how fluke avoid and/or manipulate them, and whether strategies are available to target fluke defences.

Solution Routes

What approaches could/should be taken to address the research question?

Development and availability of multiplex and NGS technologies to define regions of the host genome relevant to immune responses.

Experimental studies into host responses to infection.

Dependencies

What else needs to be done before we can solve this need?

Immunological tools for ruminants are currently limited.

State of the Art

Existing knowledge including successes and failures

Recent work has focused mainly on why protection levels from candidate vaccines is so variable, and the role of the host response in generating this variability. Other work made significant advances in understanding fluke immune avoidance and manipulation. Integration of existing knowledge into a holistic understanding of host-fluke immune interactions and new work to test this is the logical next step.

Projects

What activities are planned or underway?

Liver fluke control strategies – Lead Summary 15B (HPE Interactions – latent/carrier)

Title:	Can animals be resilient to liver fluke infection?

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Can we intervene against the “carrier state” to improve fluke control?
Is the carrier state an appropriate concept for fluke infections, in which low level infections produce eggs, which are then amplified considerably in the intermediate host, and therefore contribute actively to on-farm epidemiology and infection pressure. This is very different to the idea of carrier states in other animal diseases. Low level infections can also have production impacts. These are more appropriately defined on a continuum of fluke burden, than being falsely categorised as latent or hidden.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

The sub-clinical carrier state is dominant in fluke infections, since infection is widespread within infected herds but most animals are sub-clinically infected. Long duration of patent infection means that apparently healthy animals contribute to pasture contamination. Identification and elimination of these infections is unlikely to be any more productive than existing whole-herd chemical intervention strategies. Rather, it might be useful to determine whether animals differ in the threshold level of infection that incurs production losses, and whether this can then be manipulated to, e.g. by selective breeding.

Solution Routes

What approaches could/should be taken to address the research question?

Studies in experimentally and naturally infected animals that more precisely elucidate the relationship between fluke burden and production impacts, including intervention studies based on different indicators and treatment thresholds. Hence determine whether tolerance of infection below a given threshold is a viable strategy, and whether variation in this level between individuals and breeds is heritable and provides a route for selecting fluke-tolerant production animals.

Dependencies

What else needs to be done before we can solve this need?

Allowing persistence of the carrier state in some animals carries a danger of enabling persistent shedding of eggs into the environment and increased infection pressure across the herd and farm. This would have to be managed effectively for this strategy to be viable. There is significant danger that tolerating low levels of infection could rapidly lead to increases in parasite abundance and production impacts due to amplification in snail hosts.

State of the Art

Existing knowledge including successes and failures

To date it has largely been assumed that elimination of fluke, insofar as it is possible, is the aim of control. This is changing as existing control methods are recognised to be only partly effective, and management of drug resistance raises the question of whether generation of refugia is appropriate for fluke. This could lead to more critical appraisal of the carrier state (=sub-clinically infected individual) and management of fluke that recognises the inevitability of this state on infected farms.

Projects

What activities are planned or underway?

Liver fluke control strategies – Lead Summary 15C (Resistant/cleared)

Title:	Protective immune response to *Fasciola hepatica*

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The role of the host immune system in controlling / containing infection

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Characterising differences in the responses of sheep and cattle and between breeds of animals and individuals, in protective response after first infection.
Applying these differences to interventions that help control fluke.

Solution Routes

What approaches could/should be taken to address the research question?

Fundamental studies are needed to characterise immune responses to experimental and natural infections, how these differ between animals, and what routes are open to encouraging more effective immune responses in livestock.
An important component will be understanding of fluke evasive strategies and how these might be curtailed.

Dependencies

What else needs to be done before we can solve this need?

Improved understanding of host-pathogen interaction and the development of protective immune responses.
Better immunological tools for ruminant species.

State of the Art

Existing knowledge including successes and failures

Some differences in susceptibility have been observed (e.g. cattle refractory to repeat infections) but mechanisms and whether this is helpful in fluke control are not well known. For example, liver fibrosis in cattle might limit future patent infections but at the cost of production loss.
Understanding of immunity to fluke has built up, e.g. within vaccine trials (see 15A) but new tools and approaches have the potential to integrate this knowledge and apply it more generally to control strategies.

Projects

What activities are planned or underway?

Liver fluke control strategies – Lead Summary 16 (Coinfection)

Title:	Implications of co-infection for control of liver fluke.

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

How does infection with liver fluke affect the course of infections with other pathogens and vice versa? What are the implications for fluke control?

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Fluke infection appears to have effects on infection with and detection of several other pathogens. Deeper understanding is needed before implications are clear.

Solution Routes

What approaches could/should be taken to address the research question?

Intervention studies that introduce or eliminate liver fluke from animals and document the consequences for establishment and detection of bacterial and viral infections, and effectiveness of vaccination against them.

Characterisation of host immune responses to fluke alone and combined infections; effects of attempts to manipulate these interactions, e.g. through controlled exposure or immunomodulation.

Dependencies

What else needs to be done before we can solve this need?

Ability to run controlled infections including with regulated pathogens.

Better immunological tools to study ruminant immune responses.

State of the Art

Existing knowledge including successes and failures

Relationships have been observed between fluke infection and several microparasites, which affect infection status and outcomes, including increased susceptibility to bacterial infections (*Clostridium*, *Samonella* and *E. coli O157*) in fluke-infected animals, and impaired immune responses to bacteria and consequent low detection sensitivity (bovine tuberculosis). It is possible that these are widespread and affect control, e.g. through the efficacy of vaccination, and by inhibiting surveillance schemes. However, these implications have not been thoroughly explored to date.

Interactions between liver and rumen flukes in the definitive or intermediate hosts might affect disease outcomes and epidemiology but have been little explored to date.

Projects

What activities are planned or underway?

Liver fluke control strategies – Lead Summary 17 (Host range)

Title:	How does the range and relative competence of definitive and intermediate host species affect the epidemiology of *Fasciola hepatica*?

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*
Which snail species act as intermediate host for *Fasciola hepatica* and *Calicophoron* spp. infection and what is their transmission potential? Which wildlife species carry significant fluke burdens and should they be taken into account in epidemiology and control?

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
In each continent there are main intermediate hosts (eg *Galba truncatula* in Europe), but a large range of other potential intermediate hosts has also been reported. However, their role in fluke epidemiology is not well understood. Infections in these other species of snail may be accidental and may not always lead to the production of cercariae. There is a lack of information on wildlife reservoirs for F. hepatica, their competence and contribution to metacercariae on pasture used by domestic livestock.

Solution Routes

What approaches could/should be taken to address the research question?
Accurate identification of snails and their infection status and cercarial shedding capacities. Quantification of infection levels in wildlife definitive hosts and their potential to contaminate farmland and drive increased infection pressure and spread of resistance genes.

Dependencies *What else needs to be done before we can solve this need?*
Development and validation of specific molecular methods for snail identification and to determine infection status. Development of methods to evaluate cercarial shedding under various conditions. Development of/access to the life-cycle in snails. Provision of snail +/- cyst material is an ongoing bottleneck in fluke research.

State of the Art *Existing knowledge including successes and failures*
Infection with *F. hepatica* can be detected by observing shedding of cercariae, crushing or microscopic dissection of the snail. These methods have poor sensitivity in the early stages of infection, and it is difficult to distinguish the intra-molluscan

stages of different species of trematodes. A number of molecular techniques have been used to detect *F. hepatica* infection in snails, which have generally been found to be more sensitive than microscopic methods; however, the presence of inhibitory factors within the snails can reduce the sensitivity of PCRs, while the presence of parasite DNA does not prove the presence of viable infection. Conversely, it is important to ensure that putative trematode-specific PCR primers do not amplify snail DNA. A number of other trematode species, including those of birds and amphibians, have been isolated from *G. truncatula* including *Calicophoron daubneyi*, *Haplometra cylindracea*, *Notocotylus* spp., *Plagiorchis* spp.. Some of these trematodes have little or no published DNA sequence available, which makes it difficult to ensure PCRs are *F. hepatica* specific. Furthermore, few of the published *F. hepatica* PCRs have been validated for use with snails.

A variety of wildlife species have been shown to host *F. hepatica* and to produce eggs in their faeces, including mammals abundant on farmland such as deer, rabbits and nutria. There have been few attempts, however, to quantify egg shedding rates and to plot spatio-temporal overlap between wildlife, livestock and snail populations in order to make robust estimates of cross-transmission potential.
Should wildlife hosts be identified as a problem, possible solutions would require further research.

Projects

What activities are planned or underway?

Liver fluke control strategies – Lead Summary 18 (Host genetics)

Title:	Host genetic factors influencing *Fasciola hepatica* infection.

Research Question
What are we trying to achieve and why? What is the problem we are trying to solve?

Understand breed, species and individual differences in protective immune response to infection and other forms of physiological resistance, and implications for fluke control.

Challenge(s)
What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

While such differences have been observed, their genetic basis has not been elucidated, either in terms of mechanism or heredity. This understanding is needed in order to determine whether selective breeding or breed substitution are viable strategies to assist in control of fluke infections.

Solution Routes
What approaches could/should be taken to address the research question?

Genetic characterisation of hosts with greater apparent resistance or resilience to fluke infection.

Immunological studies of resistance versus susceptible lines. GWAS studies using breed and outbred ruminant populations to evaluate intra and interbreed resistance.

Dependencies
What else needs to be done before we can solve this need?

Improved genetic and immunological tools.

State of the Art
Existing knowledge including successes and failures

Because there appears to be little natural acquired resistance to fluke infection, there has been limited scope to map this to genetics and mechanisms.

Projects
What activities are planned or underway?

Liver fluke control strategies – Lead Summary 19 (Pathogen genome)

Title:	Liver fluke genome

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Improve description and annotation of the liver fluke genome and use it to enhance ability to address research challenges and improve control options.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Leverage advances in genomics and related technologies to better understand fluke biology and the genetic basis and mechanisms of important life history traits, defence against host immune responses, and adaptation to changing host, environmental and management conditions.

Solution Routes

What approaches could/should be taken to address the research question?

Deep sequencing and functional annotation of the *Fasciola hepatica* genome.
Integration of genomics and allied -omics technologies into studies of fluke biology.

Dependencies

What else needs to be done before we can solve this need?

Parasite genome sequence and annotation will lead to incremental improvements in capacity and interpretation and guide selection of experiments and markers.

State of the Art

Existing knowledge including successes and failures

First genome map of F. hepatica published; further annotation in progress. Comparative studies between genomes of F. hepatica and F. gigantica underway. Better descriptions of multiple gene families, their heterogeneity and implications for vaccine and diagnostic antigen preparation in progress. Incremental advances in genome resources and functional interpretation, at species and population level underway.

Projects

What activities are planned or underway?