STAR-IDAZ
International Research
Consortium on Animal Health

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

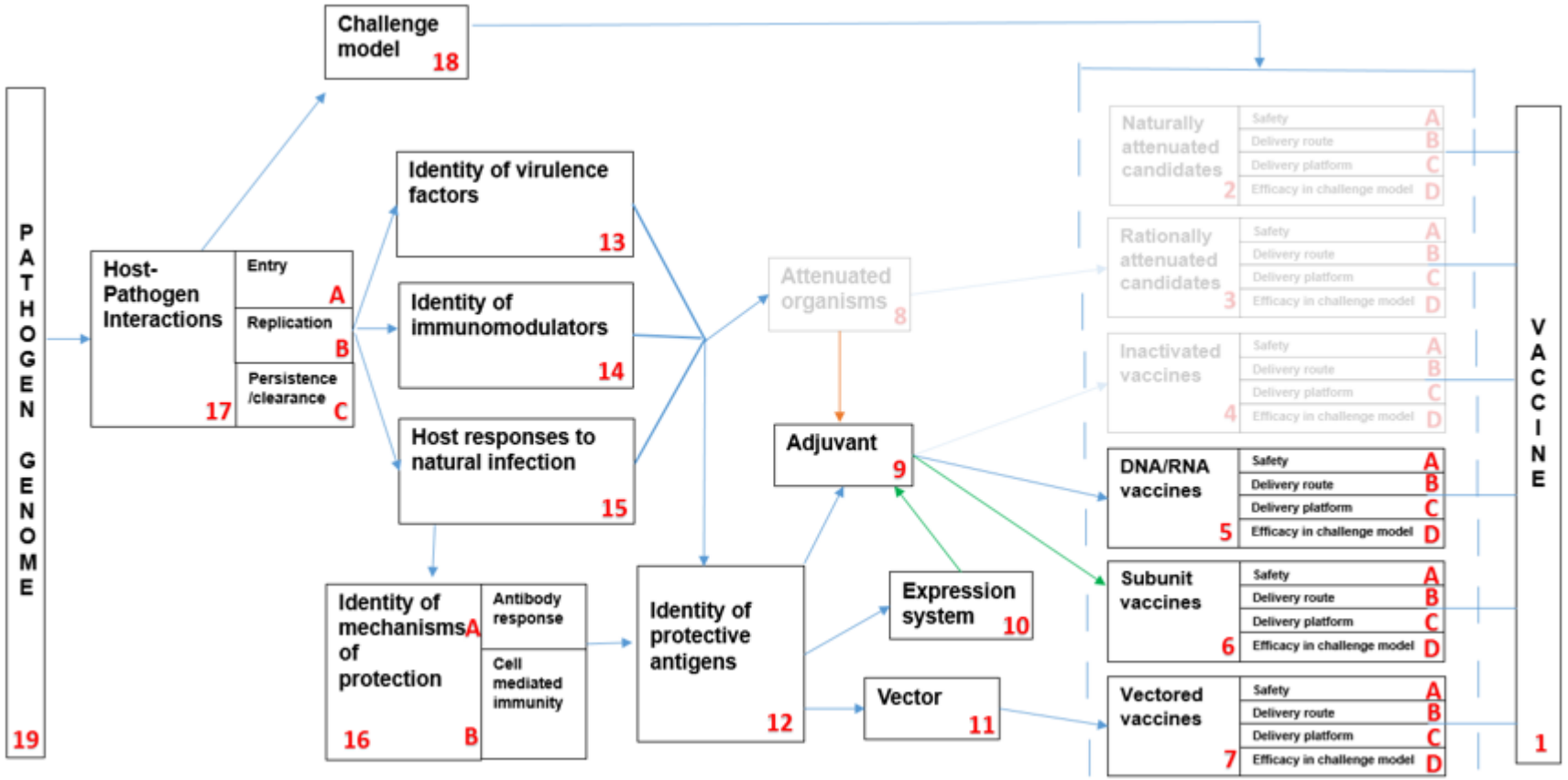
SIRCAH Deliverable 3.4

30/09/2019

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

The Secretariat for the STAR-IDAZ IRC (SIRCAH) is funded from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727494

2ai) Roadmap for the development of candidate vaccines for liver fluke



The roadmap for Trematode vaccine development has been developed by the Livestock Helminth Research Alliance (LiHRA; June 2019) with major contributions of Grace Mulcahy and Jozef Vercruysse.

Lead Summary 1

Title:	Vaccine giving long-lasting protection against infection by *F. hepatica*

Research Question

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

A vaccine giving long-lasting protection with two initial injections followed by annual boosting, ideally in both cattle and sheep

Challenge(s)

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

Need for frequent boosting where hidden antigens are used
The parasite modulates the hosts immune responses
Unknown sources of variability in extent of protection in trials with identical protocols
Need to communicate the level of efficacy likely to be delivered by fluke vaccines – will not be likely to match the 100% or near expected for many bacterial and viral vaccines

Solution Routes

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

Slow release of purified antigens from an adjuvant that drives the immune response in the correct direction.

Meta-analysis of the results of previous trials to understand the sources of variability
Continued basic immunological studies to understand fluke-host interactions and fluke-induced immunomodulation
Adjuvants capable of modulating the response to infection in a protective direction
Use of a replicating vector to reduce number of boosting doses required
Nucleic-acid based vaccines based including those based on self-amplifying RNA technology
A combination of different candidates (subunit/nucleic acid based/peptide/vectored) in a Prime-boost approach
Multivalent vaccines incorporating pathogens generating strong Th1-responses and counter-acting F. hepatica induced immunoregulation

Dependencies

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

Difficulties in agreeing measures of protection – e.g. fluke burden, egg output, liver damage, specific pathology
Modelling how much protection is required for an effective and commercial vaccine

More in-depth knowledge about host-pathogen relationships and the nature of immune response that is capable of being protective

State of the Art

Existing knowledge including successes and failures

Protection of ruminants can be achieved using a number of antigens, both purified and recombinant, derived from F. hepatica E/S molecules. However, this is inconsistent.

Possible reasons for this inconsistency include:

Genetic variation in fluke strains used for challenge
Differences in fine-specificity or other parameters of the host response
Contribution of parasite glycans to protective and non-protective responses
Host genetic background factors

Unnown unknowns!

Projects

What activities are planned or underway?

PARAGONE, and EU-funded H2020 project involving vaccine delelopment for liver fluke as well as for a range of other livestock parasites, will finish in 2019
A Science-Foundation Ireland project on basic immunology and applied vaccine science will finish in 2020
An SFI Research Professorship to J.P. Dalton at NUIG will commence in 2019
A number of national projects on fluke immunology and fluke vaccines are underway in Spain

Lead Summary 5

Title:	Development of nucleic acid-based vaccines

Research Question

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

Generation of a protective immune response against *F.hepatica*

Challenge(s)

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

DNA vaccine approach hasn't worked as well in ruminants as in rodent models.
Anticipated regulatory challenges for this approach

Solution Routes

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

DNA vaccine
Self-amplifying RNA technology
Efficacy trials of existing candidates in experimental and field challenge studies

Dependencies

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

A challenge model,
Definition of minimal acceptable efficacy
Identity of protective antigens
Identity of suitable molecular adjuvants

State of the Art

Existing knowledge including successes and failures

Very limited evidence in the target species

Projects

What activities are planned or underway?

No major projects are highlighted

Lead Summary 6

Title:	Development of subunit/peptide vaccine

Research Question

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

Generation of a protective immune response against *F.hepatica* using parasite peptides/glycopeptides giving protection for up to a year.

Challenge(s)

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

Lack of a natural boosting effect from field exposure if hidden antigens are used
Immunomodulation by field infection
Obtaining protective immune responses in the range of MHC haplotypes represented in target populations, given the limited T-cell epitopes likely to be represented in a peptide-based vaccine
Obtaining recombinants that mimic the protective capacity of native proteins in the case of a sub-unit vaccine

Solution Routes

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

Identify antigens that are physiologically important for the parasite's development and or survival – secreted or hidden.
Identify of secreted antigens that the parasite uses to modulate host responses
Produce effective recombinants and/or peptides that induce protective, but not decoy or deleterious responses

Dependencies

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

A challenge model,
Definition of minimal acceptable efficacy
Identity of adjuvants to give long lasting protective immune responses.
Identity of suitable expression vectors for large scale expression.
Identity of protective antigens

State of the Art

Existing knowledge including successes and failures

Vaccine trials are under way evaluating a range of antigens.
Excretory products have been trialled – including cathepsin L2, GST, HDM, PRx, LAP

Projects

What activities are planned or underway?

PARAGONE, and EU-funded H2020 project involving vaccine delelopment for liver fluke as well as for a range of other livestock parasites, will finish in 2019
A Science-Foundation Ireland project on basic immunology and applied vaccine science will finish in 2020
An SFI Research Professorship to J.P. Dalton at NUIG will commence in 2019

A number of national projects on fluke immunology and fluke vaccines are underway in Spain

Lead Summary 7

Title:	Development of a vectored vaccine

Research Question

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

Using a replicating organism to give enhanced exposure to protective antigens

Challenge(s)

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

If several antigens are needed to give optimal protection the vector may need to have a relatively large genome.
That the expressed antigen has the correct conformation
That revaccination doesn't result in rapid elimination of the vector

Solution Routes

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

Development of a range of genetically modified organisms expressing protective antigens of one or more parasite species either as a secreted entity or as a surface molecule

Dependencies

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

Identification of an effective and acceptable vector system
Interdisciplinary collaboration to achieve this

State of the Art

Existing knowledge including successes and failures

There have been no studies to date on vector-expressed vaccines for *Fasciola*

Projects

What activities are planned or underway?

None on this specific topic, currently

Lead Summary 9

Title:	Conventional and molecular Adjuvants

Research Question

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

Establish the identity of suitable adjuvants for conventional (an effective antigen delivery system) and/or nucleic acid-based vaccines including an effective delivery system.

Challenge(s)

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

There is comparatively little empirical knowledge on the mechanism of action of available adjuvants

In the case of newer adjuvants, many are within the IP portfolio of animal health companies

There is a need to investment in the development of "smart adjuvants" capable of targeting the qualitative aspects of immune responses

Solution Routes

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

Trial different delivery platforms

Work with animal health companies to trial proprietary adjuvants with vaccine candidates

Establish the immune response and protection levels generated using various combinations of antigens and adjuvants

Dependencies

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

Precise identity of protective immune response – Th1/Th2.

Precise Identity of protective antigens.

State of the Art

Existing knowledge including successes and failures

The analysis of a large number of trials carried out in both cattle and sheep by a number of groups independently has shown that it is possible to deliver protection of the order of 40-50% reduction in fluke burden. However, for unknown reasons, these results are variable, and some trials with identical protocols have not delivered protection.

Projects

What activities are planned or underway?

It will be very important to make efforts to understand the reasons for this variability through a metaanalysis of previous studies

Lead Summary 10

Title:	Expression system for subunit vaccine

Research Question

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

Development of an affordable stable expression system for large-scale production of the recombinant proteins/glycoproteins.

Challenge(s)

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

That the expressed antigens have the correct conformation and glycosylation
Generation of stable genetically modified organisms allowing largescale production

Solution Routes

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

Various cell line expression systems – bacteria; parasite or plant cell lines
Collaboration with commercial entities who have proprietary expression systems
Certain plant protein expression systems are capable of mimicing helminth protein glycosylation systems

Dependencies

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

Identity of the protective antigens
Collaboration between groups expert in a variety of expression systems
Detailed knowledge of the importance of parasite glycans in host-parasite responses

State of the Art

Existing knowledge including successes and failures

Quill A has been used in a number of the vaccine trials in which efficacy has been demonstrated

Projects

What activities are planned or underway?

Adjuvant studies are included in most of the projects underway at present

Lead Summary 11

Title:	Vector identification for Vectored vaccine

Research Question

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

Using a replicating organism to express the protective antigens generating a stronger longer-lasting immune response

Challenge(s)

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

Immune response against the vector

Solution Routes

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

Explore a range of possible vectors – large viruses (Herpes viruses, endogenous retroviruses), protozoa (*Trypanosoma theileri, Eimeria)*, bacteria (replication deficient such as aroA mutants)

Dependencies

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

Identity of the protective antigens
Successful antigen expression

State of the Art

Existing knowledge including successes and failures

No specific trials with Fasciola antigens as yet.

Projects

What activities are planned or underway?

None planned at present

Lead Summary 12

Title:	Identity of Protective antigens

Research Question

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

To identify antigens against which protective immune responses can be generated
The parasite must be doing something which allows it to feed and evade the host responses as it migrates through the tissues – can these be used as immunogens
Alternatively there may be factors which could be targeted such as gut antigens

Challenge(s)

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

Generation of genetically modified parasites lacking "virulence" factors or immunomodulators or use of RNAi technology

Solution Routes

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

Reverse vaccinology to identify possible protective antigens.
Establish the identity and role of putative "virulence" factors and immunomodulators by generating genetically modified parasites.
Establish which parasite genes are being expressed at different stages of feeding and their role

Dependencies

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

Improved understanding of the mechanisms of protection

(i) The role of antibody classes in protection
(ii) The role of cell-mediated immune responses
(iii) Understanding the immuno-regulatory aspects of the target parasite

Transcriptomic studies of the host response

State of the Art

Existing knowledge including successes and failures

Native fatty acid binding proteins induced 55% protection and cathepsin L1 42-69% protection in cattle.
Native leucine aminopeptidase(LAP) induced 89% protection in sheep.
Native glutathione S transferase – showed variable results in cattle and sheep
Recombinant Schistosoma mansoni 14 antigen has shown positive results in some experiments.
F.hepatica rLAP shown positive response in sheep
Recombinant versions of Cl1 and CL3 have demonstrated protection in cattle

Projects

What activities are planned or underway?

PARAGONE, and EU-funded H2020 project involving vaccine delelopment for liver fluke as well as for a range of other livestock parasites, will finish in 2019
A Science-Foundation Ireland project on basic immunology and applied vaccine science will finish in 2020

An SFI Research Professorship to J.P. Dalton at NUIG will commence in 2019
A number of national projects on fluke immunology and fluke vaccines are underway in Spain

Lead Summary 13

Title:	Identity of parasite virulence factors

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

To identify the factors that aid parasite migration and feeding. If the host could be immunised against these would it result in the parasite being unable to feed.

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

Understanding how the parasite interacts with and moves across the host intestine

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

Studies on the immune responses of naturally exposed animals to establish the direction of immunomodulation
Establish which parasite genes are being expressed at different stages and their role in parasite migration/feeding

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

An improved understanding of Host-parasite interaction

State of the Art
Existing knowledge including successes and failures

Projects
What activities are planned or underway?

PARAGONE, and EU-funded H2020 project involving vaccine delelopment for liver fluke as well as for a range of other livestock parasites, will finish in 2019
A Science-Foundation Ireland project on basic immunology and applied vaccine science will finish in 2020

An SFI Research Professorship to J.P. Dalton at NUIG will commence in 2019
A number of national projects on fluke immunology and fluke vaccines are underway in Spain

Lead Summary 14

Title:	Identity of immunomodulators

Research Question

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

To identify how the parasite modulates the host immune responses. If the host could be immunised against these would it result in the parasite being unable to migrate and be eliminated

Challenge(s)

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

Solution Routes

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

Establish which parasite genes are being expressed at different stages and their role in parasite migration/feeding
Establish the roles of Cathepsins, peroxiredoxin and helminth defence molecules in immunomodulation

Dependencies

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

An improved understanding of Host-parasite interaction

State of the Art

Existing knowledge including successes and failures

We can describe the ways in which the response of infected animals is altered, and how it affects the response to other pathogens
Modulation involves suppression of Th1 responses, induction of Th2/Treg responses, and polarisation of macrophages towards the alternatively-activated pathway.

Projects

What activities are planned or underway?

PARAGONE, and EU-funded H2020 project involving vaccine delelopment for liver fluke as well as for a range of other livestock parasites, will finish in 2019
A Science-Foundation Ireland project on basic immunology and applied vaccine science will finish in 2020
An SFI Research Professorship to J.P. Dalton at NUIG will commence in 2019
A number of national projects on fluke immunology and fluke vaccines are underway in Spain

Lead Summary 15

Title:	Host response to natural infection

Research Question

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

Identify what the host is responding to and its role in protection or possibly as a decoy dominant antigen which is preventing resistance developing

Challenge(s)

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

Establish the protective mechanisms required to kill juvenile and adult flukes
Use NGS and bioinformatic tools to map the pathways up- and down-regulated following infection
As animals do not become immon-protective une to re-infection, these must be n

Solution Routes

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

Establish the dynamics of host immune responses under field conditions.
Study the response of “Resistant” animals compared to those that are highly susceptible
Transfer studies to establish the role of antibody and immune cell populations
Studies on the immune responses of naturally exposed animals to establish the direction of immunomodulation
NGS studies of local and systemic immune responses in target species
NGS studies of animals after protective-vaccination

Dependencies

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

We are ready now to address these steps

State of the Art

Existing knowledge including successes and failures

There are recent publications on the ovine systemic and hepatic responses to infection

Projects

What activities are planned or underway?

A study of the bovine systemic transcriptomic response has been completed and ready for publication. A study of the transcriptomic changes in hepatic lymph nodes of sheep following challenge infection is underway

Lead Summary 16

Title:	Identify Mechanisms of protection against *F. hepatica*

Research Question

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

Studying the transcriptomic response of ruminants to the acute and chronic stages of infection
Identifying gene expression pathways involved in immunoregulation and whether these are open to manipulation
Identify how animals that are considered more resistant are controlling/containing the infection. What are the roles of the various antibody classes and cellular responses.
Cell types involved in the various stages of infection, including Th1/Th2 balance in "resistant" and highly susceptible animals
Identification of specific protective epitopes within putative vaccine antigens

Challenge(s)

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

The role of parasite glycans in the immune response, in immunomodulation and in the early stages of host-parasite infection

Solution Routes

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

Study the response of "Resistant" animals compared to those that are highly susceptible
Role of Molecular pattern recognition (interaction with TLRs) in determining the immune response.

Dependencies

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

A better understanding of the host-parasite interaction.

State of the Art

Existing knowledge including successes and failures

In cattle a Th1 response with high titres of IgG2 and low titres of IgG1 has been correlated with protection.
Conversely, infected animals produce specific IgG1 and almost no specific IgG2, and are not protected
A proportion of the antibody response generated in natural infection may be directed at non-protective, "decoy" antigens

Projects

What activities are planned or underway?

PARAGONE, and EU-funded H2020 project involving vaccine delelopment for liver fluke as well as for a range of other livestock parasites, will finish in 2019
A Science-Foundation Ireland project on basic immunology and applied vaccine science will finish in 2020

An SFI Research Professorship to J.P. Dalton at NUIG will commence in 2019
A number of national projects on fluke immunology and fluke vaccines are underway in Spain

Lead Summary 17

Title:	Host-parasite interactions

Research Question

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

How the host-parasite interact allowing or otherwise parasite migration, feeding and reproduction

Challenge(s)

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

How is the very early stage of infection, across the intestinal mucosa, orchestrated

Solution Routes

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

Establish which parasite genes are being expressed at different stages of feeding and their role in parasite feeding.
Establish the degree of genetic variation there is in the parasite populations and the impact of these variations on the host-parasite interaction.
Role of epithelial activation in initiating the immune responses.
Establish the importance of the different interaction of tegumental Ags and ES Ags with TLR4 and dendritic cells.
Establish the role of a range of Th2 cytokines, especially IL-25, IL-33 and TSLP
Identify the parasite proteins/Ags that modulate dendritic cell maturation
Establish the role of basophils early in infection
Establish the role of Antibody-dependent Cell cytotoxicity
Are macrophages and eosinophils targets of immunomodulation

Dependencies

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

Parasite genome sequence - annotated

State of the Art

Existing knowledge including successes and failures

In Schistosoma infected mice after an early phase of antigen specific cellular proliferation and IFNγ synthesis there is a shift to a Th2 profile with LI-4 and IL-13 production predominating and a decrease in cellular responses. By the time infection becomes patent the response is dominated by IL-10 and TGFβ and IgG1 predominates.
Initial recognition of the metacercariae takes place in the GIT.
The tegument of NEJs is primarily composed on oligomannose and core fucosylated truncated N-glycans .Ttegumatal Ag induce angeric T-cells via dendritic cells
In both cattle and sheep IgG2 are linked with the expression of resistance or protection

Projects

What activities are planned or underway?

Parasite glycan characterisation
Fluke genome projects
ERC grant
PARAGONE, and EU-funded H2020 project involving vaccine delelopment for liver fluke as well as for a range of other livestock parasites, will finish in 2019

A Science-Foundation Ireland project on basic immunology and applied vaccine science will finish in 2020
An SFI Research Professorship to J.P. Dalton at NUIG will commence in 2019
A number of national projects on fluke immunology and fluke vaccines are underway in Spain

Lead Summary 18

Title:	Challenge model

Research Question

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

What are acceptable challenge models for screening of vaccine candidates

Will we obtain different results with bolus as compared with trickle infections?

Challenge(s)

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

Is there a difference in the vaccine response to trickle (field) and bolus challenges

Can we predict differences in vaccine protection based on fluke strain

Is there a non-challenge proxy for estimating protective capacity

Solution Routes

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

Meta-analysis of previous trials used in an attempt to determine and standardise the optimum vaccine challenge model

Dependencies

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

What would regulators accept in relation to minimal efficacy

Understand the contribution of fluke genetic variability on protection following vaccination

State of the Art

Existing knowledge including successes and failures

Protective effect has been observed using both trickle, and bolus, challenge models to date. The same is true for non-protective trials

Projects

What activities are planned or underway?

PARAGONE, and EU-funded H2020 project involving vaccine delelopment for liver fluke as well as for a range of other livestock parasites, will finish in 2019

A Science-Foundation Ireland project on basic immunology and applied vaccine science will finish in 2020

An SFI Research Professorship to J.P. Dalton at NUIG will commence in 2019

A number of national projects on fluke immunology and fluke vaccines are underway in Spain

Lead Summary [*Number*]

Title:	Bystander Immunoregulation

Research Question

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

What effect does Fasciola infection have on the diagnosis and control of other livestock pathogens?
How can this relationship be utilised to enhance disease control in a holistic fashion

Challenge(s)

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

Funding for such studies which have a less obvious "end-product" than more applied vaccine studies

Solution Routes

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

Clearly communicate the utility of such studies

Dependencies

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

Incorporate sophisticated epidemiological modelling studies along with immunological insights into co-infection

State of the Art

Existing knowledge including successes and failures

F. hepatica infection has been documented as decreasing the responsiveness of cattle to diagnostic tests for bovine tuberculosis, but also decreasing bacterial load in co-infected cattle
The epidemiological significance of these effects needs to be dissected and understood.
Some evidence is beginning to be compiled concerning interaction between liver fluke and other pathogens including Mycobacterium paratuberculosis, and Calicophoron daubneyi

Projects

What activities are planned or underway?

PARAGONE, and EU-funded H2020 project involving vaccine delelopment for liver fluke as well as for a range of other livestock parasites, will finish in 2019
A Science-Foundation Ireland project on basic immunology and applied vaccine science will finish in 2020
An SFI Research Professorship to J.P. Dalton at NUIG will commence in 2019
A number of national projects on fluke immunology and fluke vaccines are underway in Spain