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

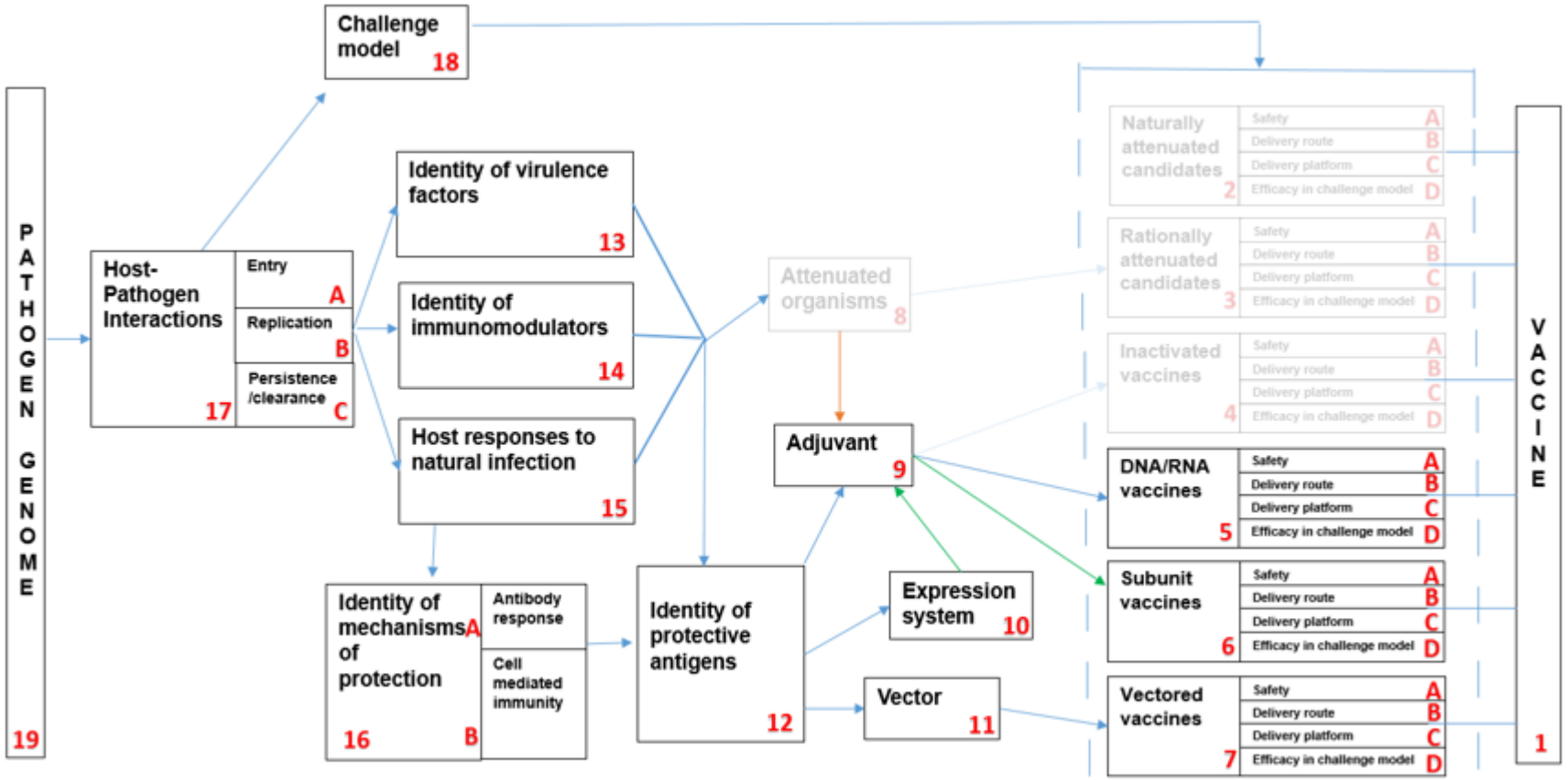
SIRCAH Deliverable 3.4

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

2bi) Roadmap for the development of candidate vaccines for nematodes



The roadmap for Nematode vaccine development has been developed by the Livestock Helminth Research Alliance (LiHRA; June 2019) with major contributions of Philip Skuce, Grace Mulcahy, Edwin Claerebout, Alasdair Nisbet and Jozef Vercruysse.

Lead Summary 1

Title:	Polyvalent vaccine giving long-lasting protection against infection by the predominant nematode species infecting livestock

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*
Development of polyvalent vaccine giving long-lasting protection against the predominant nematode species infecting livestock. Protection can be defined as reducing pathology, production losses and/or pasture contamination.

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
Multiple nematode species infecting host at same time. Variability in vaccine responses between hosts Interaction with other vaccines and/or treatments. Need for frequent boosting where hidden antigens are used. Identification of conserved protective antigens/epitopes across species and/or delivery of multiple antigens/epitopes. Parasites can modulate the host immune response. Immunological control of the different parasite species may require different branches of the immune response (e.g. systemic vs mucosal antibodies, humoral vs cell-mediated). An understanding of the host protective immune response and the antigens/epitopes that drive it.

Solution Routes *What approaches could/should be taken to address the research question?*
Purification of sufficient native antigen(s) or expression of recombinant versions to allow for commercial scale-up. Slow release of purified antigens from an adjuvant that drives the immune response in the correct direction. Use of concatenated or co-expressed antigens/epitopes. Use of a replicating vector to reduce number of boosts. Nucleic acid-based vaccines based on self-amplifying RNA technology. A combination of different candidates (subunit/nucleic acid-based/vectored) in a prime-boost approach. Collation and analysis of all relevant trial data, both successful and otherwise

Recombinant expression of protective antigens/epitopes in the correct conformation. Several different antigens may be needed for each parasite species. The different antigens may interfere with each other e.g. enzymatic degradation/inhibition.

Dependencies

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

A challenge model for species of interest.
Definition of minimal acceptable efficacy.
Identity of protective antigens.
Definition of protective immune response required for each parasite species.
Identity of suitable molecular adjuvants.
Identity of delivery platform.
Increase farmers' intention to adopt vaccination against GIN, as well as/instead of routine anthelmintic treatment.

State of the Art

Existing knowledge including successes and failures

- PP2A (recombinant part of the catalytic region of the serine/threonine phosphatase 2A) from hookworms used as polyvalent vaccine in trials to protect lambs against mixed nematode infections. Questionable efficacy (Mohamed Fawzi et al., 2013)
- Cocktail vaccine of 8 antigens for *Teladorsagia* gives up to 70% reduction in FEC (Nisbet et al, 2013), simplification of vaccine cocktail required - Co-expression of multiple antigens to simplify production (*Teladorsagia* vaccine in PARAGONE) – vaccine lost efficacy
- Native (hidden) gut antigen vaccine Barbervax in commercial production, (Smith et al., Moredun), polyvalency not confirmed
- Experimental vaccines against *Ostertagia* and *Cooperia* in cattle, based on purified native ASP antigens. Field efficacy confirmed for *Cooperia* vaccine (single experiment), not for *Ostertagia.* Recombinant expression of protective antigens unsuccessful so far.

Projects

What activities are planned or underway?

Current field trial of simplified recombinant *Teladorsagia* vaccine in sheep on pasture contaminated with multiple species (Nisbet and Kenyon, Scottish Government funded)
Recombinant expression of *Ostertagia* and *Cooperia* ASP in modified *Pichia* strain to incorporate nematode-specific glycan structures and identification of vaccine-induced immune responses against *Ostertagia* and *Cooperia* in cattle.

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?

Attempting to avoid issues (folding/conformation/post-translational modifications) of recombinant protein-based vaccines by using mammalian expression in the parasite's definitive host

Challenge(s)

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

DNA vaccine approach has not worked as well in ruminants as in rodent models.
Regulatory issues, especially for use in food animals

Solution Routes

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

DNA vaccines incorporating sequences of host cytokines to drive the immune response in correct direction
Self-amplifying RNA technology

Dependencies

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

How acceptable are nucleic acid-based vaccines for livestock i.e. food animals, GM etc.?

State of the Art

Existing knowledge including successes and failures

Bi-cistronic DNA vaccine technology incorporating cytokines well established e.g. in poultry vaccines.
Self-replicating/amplifying technology based on alphavirus machinery commercially exploited by e.g. Glaxo and Tiba Biotech.
DNA vaccine technology being used in goats for immunity against *Haemonchus* (Yan et al 2014)
Self-replicating mRNA vaccines have not yet been widely tested for efficacy against multicellular parasites (schistosomes are exception to this – Tiba Biotech have recently presented some data on this at IVVN, London 2019)

Projects

What activities are planned or underway?

None known

Lead Summary 6

Title:	Development of a native anti-parasite 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 a number of parasite species using parasite-derived (or native) proteins/glycoproteins.

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.
Several different antigens may be needed for each parasite species.
The different antigens may interfere with each other e.g. enzymatic degradation/inhibition.
Producing sufficient native parasite material for vaccine trials and commercial scale-up.
Producing consistent vaccine batches free from adventitious agents.

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 e.g. digestive enzymes – secreted or hidden.
Identify secreted antigens or those in extracellular vesicles that the parasite uses to modulate host responses.
"Fractionate and vaccinate" approach has had some success (e.g. *Haemonchus*)

Dependencies

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

Production of sufficient quantity of native antigen for trials and/or commercial scale-up. Requires consistent source of parasite material (e.g. donor animals/abattoir collection) and efficient protein extraction and purification processes.

State of the Art

Existing knowledge including successes and failures

Native protein prototype vaccines are being trialled/used for *Haemonchus* (Commercial product, Barbervax/Wirevax, launched 2014 (Smith et al., Moredun).
A recombinant Hc23 *H. contortus* antigen has shown potential in sheep (Fawzi et al., 2015, Universidad Complutense de Madrid)).
Ostertagia and *Cooperia*: Native ASP-based vaccines effective, recombinant subunit not (Geldhof et al., UGent)

Native extracts have been used in attempt to protect against *Teladorsagia* and *Trichostrongylus* with variable and/or limited efficacy (Smith et al; Emery et al, McClure et al)

Projects

What activities are planned or underway?

Larval *Haemonchus* HcL3 antigen from Piedrafita group in Melbourne still under development

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 and optimal delivery to the correct arm of the host's immune system.
Attempting to avoid issues (folding/conformation/post-translational modifications) of recombinant protein-based vaccines

Challenge(s)

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

If the same organism (vector) is to express the protective antigens of several parasites it may need to have a relatively large genome.
Production of complex antigens from multicellular organisms.
That the expressed antigen has the correct conformation.
That revaccination doesn't result in rapid elimination of the vector.
That an appropriate vector is available for the livestock species being immunised.
That the chosen vector stimulates the correct arm(s) of the immune response.
Regulatory and consumer issues regarding GMOs.
Potential pathogenicity/reversion of 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.
Epitope mapping of antigens to reduce the complexity and size of the inserted genes for expression.

Dependencies

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

Identity of a suitable vector (e.g. *Trypanosoma theileri* or autologous retrovirus).
Identity of protective antigens and their genetic sequences.
Identity of correct protective immune response.

State of the Art

Existing knowledge including successes and failures

Adenovirus and canary pox virus widely used as vectors for other pathogens.

Eimeria system in hens (Tomley et al) – some success (Pastor-Fernández et al 2018 IJP 48, 505-518
Trypanosoma system (Matthews et al., 2016) – not yet proven

Projects
What activities are planned or underway?

Sheep lentiviruses, pox viruses and herpesviruses all under current evaluation as vaccine vectors (Moredun, funded by Scottish Government Strategic Research Programme – e.g. *T. circumcincta* antigens (Nisbet et al.).

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?

Attempting to optimise the host's protective immune response to antigens from a number of parasite species.
To identify suitable adjuvants for conventional and nucleic acid-based vaccines, including an effective delivery system.

Challenge(s)

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

The protective immune response required for the different parasite species may involve different arms of the immune response and different expression/delivery systems.
Understanding the mechanism of action of adjuvants
Many adjuvants are proprietary i.e. IP still held by commercial companies

Solution Routes

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

Establish the required immune response by using information and data from previous vaccine trials with variable levels of efficacy.
Identify adjuvants and administration routes which provoke the desired response.
Establish the correct/appropriate immune response using various combinations of antigens and adjuvants.
Use of novel adjuvants with PAMPs to stimulate correct responses

Dependencies

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

Precise identity of protective antigens.
Improved knowledge of the immune mechanisms associated with vaccine induced protection.
Availability of suitable adjuvants to provoke required response in required species.
Licensing for use in livestock species/food animals.

State of the Art

Existing knowledge including successes and failures

QuilA and aluminium hydroxide gave variable results, possibly depending on the parasite species. Some use of bacterial extract/LPS in administration of Hc23, for example.

Projects

What activities are planned or underway?

Use of microcrystal carriers with adjuvanting PAMPs to stimulate Th1/Th2 type responses (McNeilly et al., Moredun)

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 recombinant protein/glycoproteins.

Challenge(s)

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

That expressed antigens have the correct conformation and glycosylation to induce protective immunity.
Generation of stable genetically modified organisms allowing large scale production.
Different antigens may require different expression systems.

Solution Routes

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

Various cell line expression systems – bacteria, insect, yeast, mammalian, parasite or plant-derived.
Epitope mapping to identify minimum required peptides for immune stimulation.
Concatamerisation of minimum required epitopes for expression.

Dependencies

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

Identity of the protective antigens for the different parasite species.
Knowledge of the importance of conformation and secondary modifications (e.g. glycosylation) for protective properties of vaccine antigens.
Freedom to operate and/or licensing of vectors and expression cell lines for commercial exploitation.

State of the Art

Existing knowledge including successes and failures

The *H. contortus* ES antigen Hc23 expressed in *E. coli* protected lambs against experimental challenge (Alunda lab, Madrid)
A vaccine cocktail of eight recombinant *T. circumcincta* antigens expressed in *E.coli* and *P. pastoris* protected lambs and ewes against a trickle challenge (Nisbet et al., 2013, 2016.)
C. elegans expression system (Britton et al., Glasgow), active proteases (H11) but non-protective vs. *Haemonchus*
Ostertagia and *Cooperia* ASPs, recombinant antigens did not protect

Projects

What activities are planned or underway?

Simplification of cocktail vaccines to allow uniformity of expression systems (e.g. rational simplification of vaccine cocktail for *Teladorsagia*)

Yeast alternative glycosylation systems (Borloo et al.) for ASPs for cattle GIN
Ongoing work to find expression systems for effective recombinants (Britton et al., *C. elegans*; Borloo et al., yeast

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 for the various parasite species, thereby generating a stronger longer-lasting immune response

Challenge(s)

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

Detrimental immune response against the vector.
Vector delivering appropriate antigens in optimal configuration to trigger protective immune response.

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 Theileria, Eimeria)*, bacteria (replication deficient, such as aroA mutants

Dependencies

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

Identity of relevant protective antigens.
Understanding the protective mechanism for the respective parasite species.
Understanding dynamics of polyvalent protective immune responses to avoid unintended consequences

State of the Art

Existing knowledge including successes and failures

None identified

Projects

What activities are planned or underway?

None planned, to best of our knowledge

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 could be generated.
The parasite must utilise gene products which allow it to establish, feed and evade host responses – can these be used as immunogens?
Alternatively, there may be factors which could be targeted such as (hidden) parasite gut antigens not recognised by the host immune system during the course of a natural infection.

Challenge(s)

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

Lack of fully annotated genomes on which to base reverse vaccinology approaches to antigen identification.
Size of predicted transcriptome/proteome from which to select antigens.
What is the basis of putative antigen selection from large genomic/transcriptomic/proteomic datasets?
Generation of genetically modified parasites lacking "virulence" factors or immunomodulators, or application of RNAi technology in parasites
Extensive genetic polymorphism in parasite populations, what are the implications for antigenic variation and vaccine development?

Solution Routes

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

Establish the identity and role of putative "virulence" factors and immunomodulators by generating genetically modified parasites or blocking them using RNAi technology.
Identify subsets of proteins on which to focus reverse vaccinology approaches.
Use convalescent/immune sera to identify antigens by immunoprecipitation or immunoscreening.
Establish which parasite genes are being expressed at different stages of the parasites' life-cycles and identify those involved in vital biological processes e.g. feeding, tissue migration

Dependencies

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

Good genome sequencing and annotation.
Improved understanding of the mechanisms of protection against the various parasite species e.g. antibody classes vs cell-mediated immunity.

Identity of parasite-derived virulence factors or factors needed for feeding.
Identity of immunomodulators.
Reliable RNAi technology for all nematode species

State of the Art

Existing knowledge including successes and failures

A native microsomal aminopeptidase (H11) and a galactose-containing glycoprotein complex form the main components of the native *H. contortus* Barbervax vaccine
Vaccination of cattle with native activation-associated secreted proteins (ASP) of *O. ostertagi* and *C. oncophora* gave a significant reduction in faecal egg count.

Rational approach used to identify the 8 antigens in the *Teladorsagia* vaccine through understanding immunomodulation and immunodominance.
Reverse Vaccinology for helminths (schistosomes, de Souza et al., 2018)
General comment that vaccine 'failures' are rarely, if ever, published.

Projects

What activities are planned or underway?

Genome sequencing projects for key livestock GIN e.g. *Teladorsagia, Haemonchus, Ostertagia (Trichostrongylus*), USDA funded.

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 establishment, feeding and survival. If the host could be immunised against these would it result in the parasite being unable to feed or establish?

Challenge(s)

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

What does the parasite use *in vivo* to perform these functions during host-parasite interaction? How does this differ between parasite species, is there any consensus?
Downregulation of responses to co-infections?

Solution Routes

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

Establish which parasite genes are being expressed at different stages of establishment, feeding and reproduction and their role in parasite virulence

Dependencies

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

An improved understanding of host-parasite interactions.
An *in vitro* culture system to mimic natural conditions
A suitable animal model for host-parasite interactions

State of the Art

Existing knowledge including successes and failures

Multiple studies to identify immunomodulators, recent niche-specific transcriptomic work (Sci Rep. 2017 Aug 3;7(1):7214.)

Projects

What activities are planned or underway?

Small RNA virulence factors (Buck et al., Edinburgh) in model nematodes and GIN of livestock (Maizels et al. Glasgow) in model species.

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 host immune responses. If the host could be immunised against these would it result in the parasite being eliminated? How does this translate across multiple parasite species?

Challenge(s)

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

As in Lead Summary 13, above

Solution Routes

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

Establish which parasite genes are being expressed at different life-cycle stages and their role in parasite feeding, establishment, migration etc.

Dependencies

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

An improved understanding of host-parasite interactions.

State of the Art

Existing knowledge including successes and failures

Area not as well developed in GIN as in trematodes

Projects

What activities are planned or underway?

Not aware of any

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 the host (protective) immune response i.e. what is the host responding to and what is its role in protection, or possibly as a decoy dominant 'smokescreen' antigen preventing protective immunity from developing.
Identify how animals that are considered more resistant or resilient to GIN are controlling/containing infection.

Challenge(s)

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

Lack of immunological reagents to fully identify effector mechanisms of host immune response.
Lack of good genomic/transcriptomic data for some species for subsequent proteomic identification of immunodominant proteins.

Solution Routes

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

Study the immune response of "resistant" and "resilient" animals compared to those that are highly susceptible.
Use of serial biopsy to establish transcriptomic response in host to help identify effector mechanisms.
Identify upstream mechanisms of antigen recognition, antigen presenting and induction of acquired immune responses (bridging innate and acquired immunity).

Dependencies

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

A better understanding of host-parasite interactions.
Good models of the induction of natural immunity.
Development or enhanced availability of immune reagents for livestock species

State of the Art

Existing knowledge including successes and failures

Many studies on the induction of natural immunity dating back to the 1950s; recent studies on the transcriptomic response to parasitism (Hopkins et al.) and on the use of effector antibodies to identify protective antigens by 2D immunoblotting and immunoaffinity purification

Projects

What activities are planned or underway?

Serial biopsy of sheep abomasum during trickle infection for transcriptomic analyses (Moredun/University of Glasgow - McNeilly and Babayan)

Lead Summary 16

Title:	Identify mechanisms of protection

Research Question

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

Understanding gene expression in both host & parasite over course of infection
Identify differences between immune responses induced by protective versus non-protective vaccines (e.g. protective native vs. non-protective recombinant vaccine or protective vs. non-protective adjuvant).
What are the roles of the various antibody classes and cellular responses? Does driving a Th-2 type response benefit the parasite?

Challenge(s)

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

Multiple parasite species in natural infections, different timings & seasonality, possibly different mechanisms of protection, different sites within the host, possibly interacting with other species/co-infections etc.

Solution Routes

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

Study the response of “resistant” and “resilient” animals compared to those that are highly susceptible.
Role of molecular pattern recognition (interaction with TLRs) in determining the immune response.
Cell types involved in the various stages of infection, including Th1/Th2 balance.

Dependencies

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

A better understanding of host-parasite interactions.

State of the Art

Existing knowledge including successes and failures

In general, Th-2 type responses are generated against GI nematodes

Projects

What activities are planned or underway?

Some ongoing vaccine work funded through Scottish Govt Strategic Research Programme (Nisbet, McNeilly et al.)

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 and parasite interact, allowing or inhibiting parasite establishment, feeding and reproduction

Challenge(s)

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

Establishment of the neuroendocrine mechanisms of parasite-induced inappetance.

Solution Routes

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

Establish which parasite genes are being expressed at different stages of the life-cycle and their role in parasite feeding, establishment and reproduction.
Establish the degree of genetic variation in parasite populations and the impact of these variations on the host-parasite interaction and implications for vaccination.

Dependencies

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

Good quality accessible annotated parasite genome sequences.
Model systems for each species and relevant co-infections.
Reagents to dissect the immune responses (+/- protection)

State of the Art

Existing knowledge including successes and failures

Ostertagia/Teladorsagia/Trichuris influence protein digestion and utilisation.
Adequate knowledge of many systems for single species infections, paucity of studies/data on co-infections (helminths + other pathogens)

Projects

What activities are planned or underway?

Some ongoing work as by-product of vaccine studies, SG-funded (Nisbet, McNeilly et al.)

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 e.g. *in vitro* vs *in vivo*?

Challenge(s)

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

Complexities of the immune response and protection mechanisms during a trickle challenge as compared to a bolus challenge.
Trickle challenge is better but usually doesn't involve >1 species so different to field situation.
Co-infection with other (potentially immunomodulatory) pathogens is usually excluded in the challenge model but will be present in field situation.
What happens if animals are treated with anthelmintic
Different grazing management in different regions (e.g. winter housing vs. permanent grazing) needs to be accounted for (e.g. by field trials in different management systems).

Solution Routes

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

Always performing challenge in livestock model, (avoiding/supplementing mouse models).
Perform single and multi-species challenges but have the tools to identify species-specific responses.
Model responses based on empirical data.
Comprehensive analysis of all vaccine studies, successful and otherwise

Dependencies

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

What would regulators accept in relation to minimal efficacy
To what extent would regulatory authorities accept model data
Understanding role of parasite strain and inherent genetic variability at population level

State of the Art *Existing knowledge including successes and failures*
Good mono-specific models established for trickle infections of *Ostertagia, Cooperia, Teladorsagia, Trichostrongylus*

Projects *What activities are planned or underway?*
Lack of studies, few establishments with appropriate facilities and expertise, no known co-infection work