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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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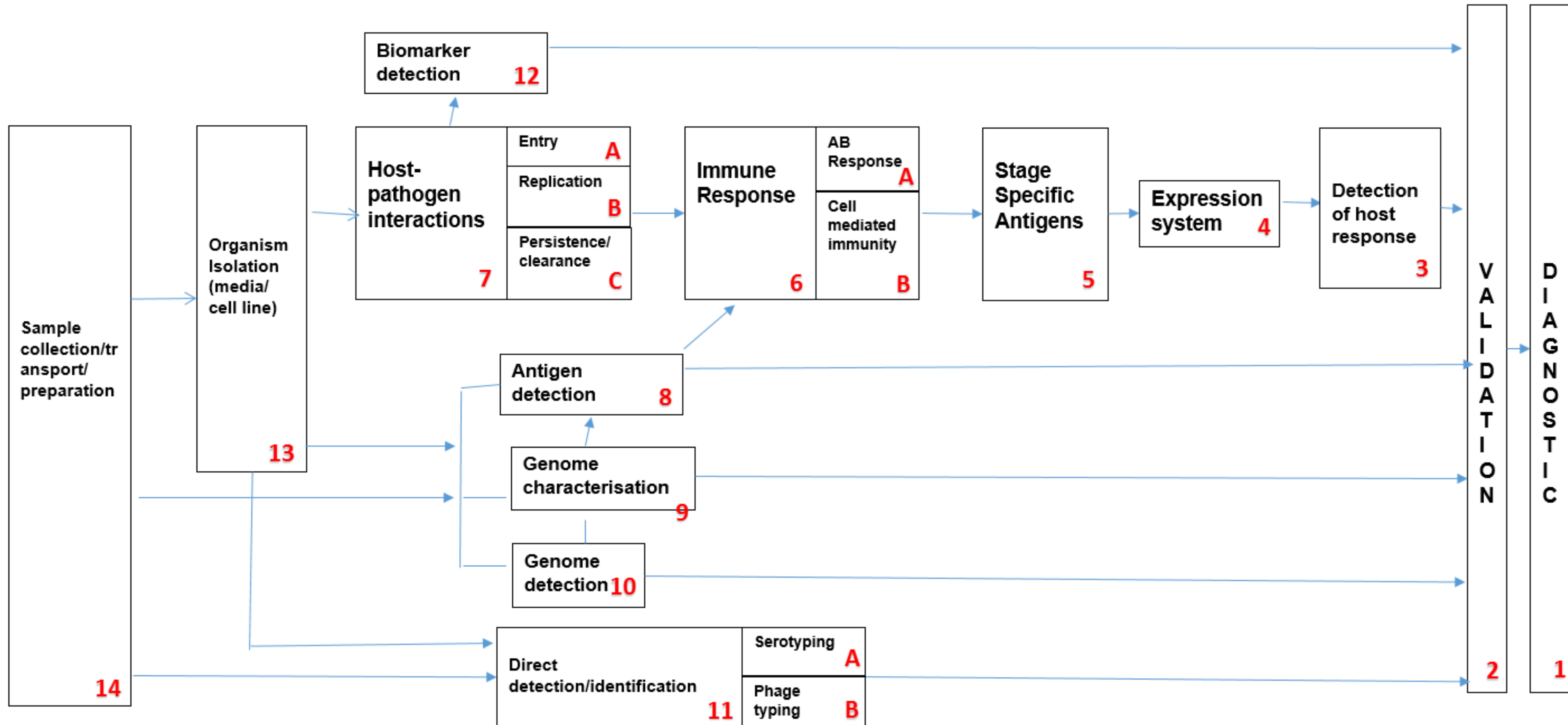
1a) Roadmap for development of diagnostic tests for helminths



The Diagnostic test roadmap has been developed by the Livestock Helminth Research Alliance (LiHRA; June 2019) with major contributions of John Gilleard, Georg von Samson Himmelstjerna, Diana Williams, Laura Rinaldi, Edwin Claerebout, Peter Geldhof and Jozef Vercruysse.

Lead Summary 1

Title:	Improved diagnostics for nematode and trematode infections in ruminants

Research Question

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

Rapid, cost-effective, pen-side and/or laboratory-based diagnosis of infection, including the level of infection and morbidity and the presence of AR, threshold for economically relevant infection levels to enable targeted and evidence-based treatment and control strategies.

Challenge(s)

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

1. Lack of accurate, sensitive,rapid and cost effective tests to **quantify** herd level infection intensities, morbidity, production impacts and anthelmintic resistance status of fluke and GI nematode infections

2. Inability to easily identify individual animals needing treatment or other interventions

3. Lack of rapid tests for acute disease syndromes or risks. eg. acute fluke infection in sheep or inhibited nematode larvae

4. Economic constraints due to low revenues in livestock particularly small ruminant farming and thus only very limited financial capacity for diagnostics

Solution Routes

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

1. Develop biomarkers (parasite and host) such as liver proteins, regulatory hormones, secreted parasite molecules for diagnosis on non -invasive sample matrices.
2. Develop molecular genetic tests for drug resistant parasites
3. Develop scalable, rapid and affordable diagnostic platforms for laboratory-based and penside tests.
4. Develop sensors and wearable technologies that can be implanted directly in or on the animals or in the environment (barn or pasture)
5. Develop genetic tests to assess the susceptibility/resilience of individual animals
6. Establish meaningful/informative group or flock diagnostic approaches
7. Further develop automated coproscopic analysis tools

Dependencies

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

1.Improve genomic resources and diagnostic platforms for both host (i.e. genetics) and helminths
2. Improve understanding of host immune responses to helminths (including antigen and antibody identification)
3. Improve understanding of molecular mechanisms of drug resistance
4. Improve understanding of appropriate diagnostic platforms
5. Develop criteria and methodologies for rigorous validation of tests

State of the Art

Existing knowledge including successes and failures

Accurate, quick and simple quantitative diagnosis of helminth infection is needed to allow evidence-based parasite control, targeted treatments, better surveillance, monitoring and anthelmintic stewardship. However most current diagnostics depend on microscopic detection of eggs or helminth-specific antigen in faeces, or detection of antibody in serum or milk. All of these assays have limitations in accuracy and sensitivity and results are normally only available to farmers several days after the sample has been collected. All these assays have specific limitations, for example, antibody tests may detect historic infection. Furthermore, as individual animal tests they are usually to costly for routine infection monitoring. Significant advances have been made in the development of web-based and semi-automated coproscopic tools.

Projects

What activities are planned or underway?

Lead Summary 2

Title:	Validation of diagnostic tests

Research Question

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

Current tests have a variety of deficiencies including accuracy, specificity, sensitivity, speed, cost and significant requirements for labour and /or specialist technical expertise. This limits their practical application and routine uptake in the field.

Challenge(s)

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

1 Need to validate biomarkers for infection intensity, morbidity or production impact for fluke or nematodes.
2. Need to validate anthelmintic resistance mutations and validate genotype to phenotype relationships in fluke and nematodes
3.Need to further validate pooling and composite sampling approaches
4. Need validation of species-specific quantitative diagnostics required Eg rumen fluke and liver fluke, different nematode species
7. Need to validate much more usable (e.g. less costly) diagnostic platforms including wearable technology, penside tests and laboratory tests,.
8. Need to better understand clinical relevance of different levels of anthelmintic resistance for the various helminth species
9. Agree and adopt approved methods for validating new diagnostic tests to establish diagnostic sensitivity, specificity, values using field studies, positive and negative predictive values and appropriate diagnostic cut off values. For this, validated panels of samples from mono-species infected animals are required.

Solution Routes

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

WAAVP approved guidelines for validating diagnostic assays in the absence of a gold standard.
A panel of samples from animals mono-infected with helminth species to test for cross reactivity.

Dependencies

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

Diagnostic tests need to be supported by decision trees to help farmers interpret their results and administer drugs or implement control programmes effectively.

State of the Art

Existing knowledge including successes and failures

1. A range of serological tests have been developed, some also as commercially available tests (e.g. Ostertagia-, Fasciola-ELISA)

2. Data on use of pooled faecal samples for group/flock coproscopical diagnosis have been published
3. A variety of molecular tests for nematode species identification (RT-PCR, pyrosequence genotyping, next-gen sequencing based methods). Largely confined to research use at present due to complexity and cost.
4. Several molecular tests for the analysis of benzimidazole-resistance associated beta-tubulin allele frequencies in pooled trichostrongyle larvae DNA samples have been developed which are suitable for field use. Largely confined to research use at present due to complexity and cost.
5. Currently, there are no validated molecular tests for resistance mutations for other drug classes.

Projects

What activities are planned or underway?

Lead Summary 3

Title:	Detection of host responses

Research Question

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

To develop diagnostics based on host responses that:
1.Assess the infection levels, morbidity and/or production impact of helminth infections to guide selection of treatment or control choices.
2. Identify stage of infection (acute, chronic, prepatent)
3 Identify resistant or resilient hosts

Challenge(s)

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

1.Incomplete knowledge of host immune and immunoregulatory responses to helminth infections, how they vary during the course of infection and how they correlate with the infection intensity and production impacts (see section 6A for more details).
4. Correlation between host responses and the levels of infection/production impacts may not be linear.
5.Immune markers may reflect historic infection. It is difficult to differentiate between current and historic infection.
6.Incomplete information available on dynamics of host responses in relation to stage of infection. e.g. juvenile versus adult infection in fluke, hypobiotic larvae).
7. Cross-reaction (lack of specificity) between parasite antigens and species, especially when using crude antigen mixtures or native antigen preperations.
8. Thresholds for economic impacts need to be understood which is complex based on regional and production system differences.
9. Non invasive sampling required eg, milk, saliva

Solution Routes

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

1. More complete descriptions of immune responses and immunoregulatory responses in hosts to helminth infections in experimentally and naturally infected animals (more details in section 6A).
2. Development of quantitative tests to measure level of antibody in relation to parasite load.

3. Develop diagnostic techniques for simultaneous (quantitative) detection of (immune responses against) multiple parasites (e.g. Luminex)
4. Investigate the use of non-invasive, user-friendly matrices to measure immune responses (e.g. copro-antigen, milk antibodies, saliva antibodies). Including for rapid penside tests such as lateral flow devices and biosensor chips.
5. Determine frequency distribution of immune parameters, determine minimum sample size to obtain results that are useful at herd/flock level and minimum number of animals that need to be positive to obtain a positive bulk/pooled sample
6. Analysis to identify evidence of cross reactivity of host antibody to a specific parasite antigen and antigens of other helminth species.

Dependencies

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

1.Improve knowledge of the specific immune and physiological responses of the host to various nematode and trematode species, including the antibody response to different parasite life cycle stages (section 6A).
2. Identify and characterise antigens and other parasite molecules that elicit host responses.
3. Improve antibody detection technologies, including isotype specific reagents for each host species's amplification systems to improve sensitivity; epitope mapping of to improve specificity.

4. Develop technologies for penside and/or quantitative detection of (multiple) parasite-specific immune responses, using non-invasive matrices
5. Identify end user expectations for parasite diagnostics and incentives/barriers for uptake of diagnostics by end users.
6. Select the best parasitological and/or production parameters to identify resilient animals

State of the Art

Existing knowledge including successes and failures

A range of helminth antigen specific antibody detection assays are available using a range of platforms and for a variety of media (serum, milk, bulk milk).
Commercial copro-antigen ELISA tests available for *Fasciola hepatica*

Projects

What activities are planned or underway?

Lead Summary 4

Title:	Development of expression systems for the large-scale cheap production of test antigens

Research Question

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

1.The production of recombinant parasite antigen for inclusion in diagnostic tests
2.Efficient and reliable production of target species- and stage-specific antigens to allow sensitive, specific and quantitative diagnosis.

Challenge(s)

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

Expression systems are needed that will provide high yields of soluble antigens with appropriate conformation and post-tralational modifications with respect to native antigens.

Solution Routes

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

1.Expression systems that preserve the conformational structure and post-tranlational modifications of the native antigen
2.Expression systems that allow large scale production of parasite antigens
3.Expression systems that produce high yields of soluble antigen in a recoverable form.
4.Production of reproducible batches of expressed protein/peptide.

Dependencies

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

The identification of appropriate antigens for testing in expression systems. This in turn requires good genomic resources (well assembled and annotated genomes) for target helminth species, an understanding of the complexity of relevant gene families, and genetic polymorphisms of antigen genes.

State of the Art

Existing knowledge including successes and failures

For fluke, several recombinant antigens have been produced and one in particular, a mutant CL1 (lacking the active site) is used widely in diagnostic tests. But not commercially available, not available as a penside form for ruminants.
For nematodes some recombinant (e.g. Teladorsagia, Dictyocaulus) as well as native (ES) antigens (Ostertagia ostertagi) have been successfully employed in different ELISA formats (serum, milk)

Projects

What activities are planned or underway?

Lead Summary 5

Title:	Identification of stage-specific antigens that can be used in immune tests

Research Question

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

1.Establish if an infection is recent or chronic using antigens that are specific for different stages of the parasite
2.Detect pre-patent infections and/or infections with pathogenic versus non-pathogenic stages.

Challenge(s)

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

1. Incomplete knowledge of stage-specific antigens of the different helminth species.
2. Difficult to obtain specific different parasite stages from hosts to undertake research to identify stage-specific antigens.
3. Better genomic and proteomic resources needed to identify stage specific antigens.
4. Complexity of expression patterns of different antigens which may include variance with immune status and other phylsiological effects.

Solution Routes

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

1.Detailed transcriptomic and proteomic analysis of stage specific antigens from each parasitic stage of the major helminth species.
2. Detailed analysis of secretomes of different parasite stages including episome analysis under in vitro and in vivo under different immune and physiological conditions.
3. Detailed characterisation of humoral and cellular immune responses to stage-specific antgens.
4. Detailed biochemical analysis of parasite extracts that are currently used in diagnostics to identify individual antigens that could be more species- and/or stage-specific.

Dependencies

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

1. High quality reference genomes for major target helminth species.
2. Better fundamental knowledge of helminth biology and host-parasite interactions,
3. Better knowledge of the immune responses to various helminth stages

State of the Art

Existing knowledge including successes and failures

Projects *What activities are planned or underway?*

Lead Summary 6A

Title:	A knowledge of the antibody response to infection with the various parasite species including its temporal nature

Research Question

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

A better understanding of the antibody responses to the various parasite species and antigens at different stages of infection and how this relates to infection intensity, disease and production impacts. This is needed to underpin the development of diagnostics based on the detection of host antibody responses responses.

Challenge(s)

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

1.Incomplete knowledge of immune responses and immunoregulatory responses in hosts to different helminth infections.
2.Incomplete understanding of how immune parameters vary during the course of infection (such as antibody isotype switch to inform choice of detection system).
3. Incomplete understanding how antibody responses and cellular responses correlate with intensity of infection, pathology, production impacts or host resilience.
4.Incomplete information available on dynamics of host responses in relation to stage of infection. e.g. juvenile versus adult infection in fluke, hypobiotic larvae).
5. Incomplete understanding of specifity of antibody responses to different species and stages of helminths.
6. Lack of reagents to probe antibody responses for target livestock species. Eg. Isotype specific antibodies

Solution Routes

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

1. More complete descriptions of host immune and immunoregulatory responses to helminth infections in experimentally and naturally infected animals.
2. Full characterisation of immunoglobulin isotype responses, to specific parasite antigens, linked back to the type of immune response induced during infection.
3. Better understanding of the rate of decay of parasite specific immunoglobulin molecules post-treatment or parasite expulsion.
4.Identification of immunogenic stage specific antigens that elicit detectable antibody responses.
6.Assess quantitative relationships between antibody responses and infection levels and parasite induced production losses. Determine thresholds for clinical or economically relevant infection levels.
7.Study seasonal and yearly (climate driven) infection dynamics and determine optimal timing for sampling, to detect or predict infection levels and/or associated production losses.
8.Large scale epidemiological studies to determine between and within-herd spatio-temporal variability in parasite infections and parasite specific host immune responses

Dependencies

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

Better reagents, tools and techniques for investigating the antibody responses of target livestock species.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 6B

Title:	A knowledge of the cell-mediated immunity to infection with the various parasite species including its temporal nature

Research Question

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

Most helminth infections elicit T helper cell type 2 immune responses These cellular responses are important, both in orchestrating the B cell response and in eliciting potentially protective immune responses.The research question is, could cell-mediated immune responses be used as a more accurate alternative to antibody responses for detection of helminth parasite infection? For example, using the interferon γ response of T cells in response to parasite specific antigen and B cell ELISPOT assays for specific/quantitative tests.

Challenge(s)

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

1. Incomplete knowledge of cellular immune responses during helminth infection
2. Incomplete understanding of immunoregulatory responses induced by parasite infection and how they may affect detection of parasite specific responses.
3. Lack of technologies that could be used to detect specific cellular responses for diagnostic purposes in target livestock species.
4. Animal to animal variation in cellular responses.
5. Incomplete knowledge of temporal development of cellular immune responses over the course of an infection, particularly in naturally exposed animals between different parasite stages including immature and and inhibited stages.
6. Lack of tools/reagents to probe cellular immune responses in target livestock species.eg recombinant cytokines, cytokine specific antibodies

Solution Routes

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

1. Analysis of parasite specific cellular immune responses following both experimental and natural infection
2. Description of the of immunoregulatory responses induced by parasite infection and how these affect detection of parasite specific responses.
3. Development of rapid, user friendly technologies that could be used to detect specific cellular responses for diagnostic purposes.
4. Investigation of immune responses in populations of animals naturally exposed to infecton to quantify animal to animal variation in cellular responses.
5. Development of a panel of stage specific antigens that can be used to differentiate between early and chronic infections and developing and inhibited larvae.

Dependencies

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

1. Development of defined, repeatable challenge models/systems under experimental and natural challenge to define cellular immune responses, immunoregulatory responses and stage specific responses in vivo/ex vivo.
2. Development of tools to probe cellular immune responses in target livestock species.
3. Panels of defined stage specific antigens.
4. Population level studies (GWAS) to identify and quantify animal to animal variation in response to helminth parasites.

State of the Art

Existing knowledge including successes and failures

A range of experimental challenge models have been developed for the major helminth species. However field level exposure systems are much less well developed. Field challenge experiments depend on conditions in a particular year (e.g for natural fluke challenge experiments) or level of contamination of pasture.

There are few examples of stage specific antigens to which cellular immune responses have been well characterised.
There are few examples of GWAS for parasites in ruminants.
There are few examples of cellular markers that are used for diagnosis.
The bovine tuberculosis interferon γ is one of the few examples on the market.
Technology lacks behind that of antibody detection tests.

Projects

What activities are planned or underway?

Lead Summary 7A,B and C

Title:	Host-pathogen interaction - entry and persistence

Research Question

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

1.To better understand the biological and immunological processes, and associated molecular and proteomic changes, that happen during early infection. This may help the development of earlier infection detection tools to eneable intervention before the onset of clinical disease.
2.To understand the factors leading to persistent infection such as entry and exit from hypobiosis in the host.

Challenge(s)

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

1.Incomplete genomic/proteomic resources and data for most target parasite and host species.
2. Controlled single species experimental infections need to be performed and appropriate experimental infection models often not well established.
3.Experiments expensive and research funding in this area difficult to obtain.
4. Immunological tools and basic reagents are generally limited for target species.
5. Considerable inter-individual variability and thus need for comparatively large experimental groups and replicates
6. In the field co-infections are common with associated interactions and clinical consequence

Solution Routes

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

Take advantage of studies going on for other purposes e.g. registrational trials or routine passaging of parasite isolates.

Dependencies

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

1.Improve genomic/transcriptomic/proteomic data on various parasite species.
2.Develop good experimental in vivo infection models.
3. Develop in silico infection models for co-infections

State of the Art

Existing knowledge including successes and failures

Existing knowledge predominantly based on studying a small range of major helminth species (eg, Fasciola, Ostertagia , Haemonchus) with much less work on mixed infections under field conditions.

Projects

What activities are planned or underway?

Lead Summary 7B

Title:	Host-pathogen interactions - replication

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

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?*

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

State of the Art *Existing knowledge including successes and failures*

Projects *What activities are planned or underway?*

Lead Summary 7C

Title:	Host-pathogen interactions –persistance/clearance

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

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?*

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

State of the Art *Existing knowledge including successes and failures*

Projects *What activities are planned or underway?*

Lead Summary 8

Title:	The detection of parasite specific antigens using antigen capture techniques incorporated in a diagnostic platform

Research Question

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

To develop cost effective, multispecies, quantitative and laboratory-based and point-of-care assays to detect antigens excreted from the host during helminth infection in biological samples? Eg faeces, saliva, urine, milk.

Challenge(s)

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

1.Lack of fundamental knowledge of the parasite and host antigens excreted from the host at different stages of infection for the different helminth species.

2.Need for there to be a quantitative relationship between excreted antigen(s) and helminth infection intensities and/or disease and production impacts.

3. Antigens need to be species- and stage-specific

Solution Routes

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

1. Development of antigen capture / immunoassays to detect helminth species and stage- specific antigens excreted in biological material convenient for sampling eg. faeces, milk, saliva etc.
2. Develop protein-biochemistry detection tools such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) profiling to allow multi-species detection and differentiation by the analysis of a single crude protein extract.
3. Investigate helminth molecule classes, other than antigens, excreted from such as microRNAs, DNA, exosomes, EVs and metabolites in circulation or faeces.
4. Development of point of care diagnostic platforms for antigen detection

Dependencies

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

1.Well annotated and assembled reference genome sequences and proteomic datasets for antigen mining and characterisation

2.Fundamental research on antigens and other molecules secreted by the different helminth species and relased from the host such as

proteins, peptides, extracellular vesicles, microRNAs, DNA and metabolites in circulation or faeces
3.Development of optimal sample preparation methods.

State of the Art

Existing knowledge including successes and failures

An ELISA coproantigen detection assay for Fasciola spp is commercially avalaible which can detect adult worm infections but is less reliable for immature pre-patent stages. There has been less progress in coproantigen detection in nematodes of livestock although there are commercially available coproantigen detection tests for companion animal nematodes such as Toxocara, Trichuris and hookworms. These have somewhat greater sensitivity that egg detection by fecal flotations and are more scalable.

Projects

What activities are planned or underway?

Lead Summary 9

Title:	Genome characterisation for improved diagnosis

Research Question

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

Improve parasite genomic, transcriptomic, proteomic and metabolomic resources and methodologies to underpin the identification of biomarkers for infection intensity and genetic markers for anthelmintic resistance diagnostics.

Challenge(s)

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

1.Helminths have complex genomes and high levels of genetic variation which makes genome assembly, annotation and analysis challenging.
2. Incentive for researchers to continue to improve genome assemblies and annotations beyond first published drafts is low since publication impact is low for "genome improvement papers".
3. Ongoing curation of reference genomes, genomic data and associated informatic resources and tools is dependant on individual researcher interest/goodwill.
3.Relatively few resources or research to date for novel helminth biomarker discovery (microRNA, metabolites etc)

Dependencies

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

Expansion and improvement of current draft genome assemblies for livestock helmith species and sustainable systems for data curation.

State of the Art

Existing knowledge including successes and failures

1.Many draft reference genomes now available for an increasing number of livestock nematode and trematode species. However, still a number of species without such respources.

2. Most of the current draft genome genomes are not sufficiently complete (both in terms of assembly and annotation) to allow genetic mapping/population genomic approaches to identify anthelmintic resistance loci or to allow truly comprehensive whole genome approaches. Haemonchus contortus is still the only chromosomal level reference genome assembly to date

4.Chromosomal level genome assemblies are needed for genetic mapping and population genomic approaches to identify genetic loci for drug resistance markers.
5. Many labs working on helminth diagnostics do not always have access to ppecialist bioionformatic expertise needed to mine and use genomic data.
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Solution Routes

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

1. Continued Improvement of current reference genome assemblies and using short read and long-read technologies
2. Generate reference genomes for those helminth species lacking such a resource, eg some of the cattle and sheep GIN species.
3. Continual improvement of annotations for the major parasite species of interest.
4. Provide web-based publically available curated databases and associated tools for the reference genomes.
5. Develop platforms to allow continued improvement of gene annotation and information by the helminth research community eg. WebApollo.
6. Undertake population genomic and genome-wide approaches to investigate and define genetic variation in relevant helminth species and identify anthelmintic drug resistance loci.
7. Undertake more discovery research on cell-free DNA, microRNA, proteomics, metabolics for biomarker and resistance marker discovery.

Lead Summary 10

Title:	Quantitative molecular tests for the detection of specific parasite species and anthelmintic resistance in practically useful diagnostic platforms

Research Question

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

The increasing levels of anthelmintic resistance (AR) in many helminths is leading to an urgent need to move towards evidence-based anthelmintic use and have better anthelmintic stewardship.
This requires quick, accurate, sensitive and cheap molecular tests in order to:
1. Quantify the DNA or RNA from different helminth species present in fecal samples before and after drug treatments.
2. Detect AR mutations for each of the major anthelmintic classes in each of the parasite species of interest using parasite DNA isolated from faeces.
3. Provide farmers and veterinarians with an assessment as to the clinical consequences/production impacts of parasite infections and anthelmintic resistance status present in the helminth population on their farm.

Challenge(s)

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

1 Levels of parasite DNA (or RNA) in fecal samples may not reflect adult helminth burdens and may not detect pre-patent/immature parasite stages.

2.High volumes of feces may need to be processed to obtain required sensitivity and mitigate effects of parasite aggregeation in fecal samples.
3.Presence of parasite stages or "free DNA" ort RNA in feces is variable. It will be determined by the physical location of the parasite, eg for Fasciola, immature stages are in the liver parenchyma so parasite DNA unlikely to be present in faeces.
4.Molecular genetic basis of resistance to different classes of drug in each different species is incomplete and molecular markers still lacking.
5. Accurate quantitation of the frequency of resistance mutations (and the different species) in helminth populations needed (rather than simple presence or absence)
6. Diagnostic platforms, either lab-based or penside, need to be sufficiently rapid, cheap and scalable to provide meaningful and sufficiently timely information for field use.

Solution Routes

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

1.Laboratory based tests or penside tests both have potential roles in providing timely information at the herd level for surveillance, monitoring, anthelmintic stewardship and herd/flock health planning.
2. Further development and validation of molecular markers and Next-generation sequencing approaches for species differentiation of eggs and larvae in fecal samples.

3. More work to identify the major genetic mutations (markers) underlying AR for the different drug classes for the major parasite species of interest: includes genetic , mapping, population genomics and functional genomics approaches.
4. More field based research piloting molecular diagnostic approaches to establish proof of concept.

State of the Art

Existing knowledge including successes and failures

1.Accurate, quick and simple quantitive diagnosis of specific helminth species and AR mutations would improve surveillence, monitoring and anthelmintic stewardship. Several PCRs have been described but they have not transitioned to diagnostic use and are only available in specific laboratories because specialized equipment needs and costs. There are also problems in reproducibility between laboratories, with published methods often not working in other diagnostic laboratories.
2. There has been recent progress in applying next -generation amplicon sequencing technologies for quantitation of helminth species in fecal samples (eg. Nemabiome ITS-2 rDNA sequencing) and of benzimidazole resistance mutations in cattle and sheep nematodes but these require more validation in the field.
3. Good understanding of the most important mutations underlying benzimidazole resistance in cattle and sheep trichostrongylid nematode species. Recent progress in genetically mapping mutations underlying ivermectin resistance, levamisole resistance and monepantel resistance in Haemonchus controtus. This should allow more proof of concept field diagnostic studies to be performed,.

Projects

What activities are planned or underway?

Lead Summary 11

Title:	Improved faecal egg counting methods for direct detection of helminth infections and anthelmintic resistance

Research Question

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

1.Improve rapidity,sensitivity,accuracy and repeatability of fecal egg count methods for the various helminth infections of veterinary importance that enable sustainable parasite control methods such as targeted (selective) treatment approaches.
2..Update guidelines for FEC/FECRT performance and interpretation.

Challenge(s)

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

1. FEC methods are labour intensive, costly and so insufficiently used for infection monitoring.
2. Quality assurance between different operators and labs is difficult
3. FEC in some host species such as for cattle nematodes and fluke are insensitive and inaccurate measures of infection intensities.
4. Liver fluke faecal egg counts need further validation for composite samples.
5. Use of composite samples in liver fluke FECRT other than for triclabendazole in sheep.

Solution Routes

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

1.Develop, deliver and disseminate the use of pooled samples for infection and drug efficacy monitoritnfg
2.Development of field-applicable kits for FEC/FECR tests to quantify helminth infection, anthelmintic efficacy and AR.
2. Development of automated systems for FEC/FECRT.
3.Development of Smartphone technologies and Apps for FEC and FECRT

Dependencies

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

Development of an image-analysis software able to identify and count helminth eggs in order to reduce the time required for the analysis and the human errors.

State of the Art

Existing knowledge including successes and failures

In an era of technological revolutions in the diagnostic industries, diagnostic methods for parasitic helminth infections are also

evolving, for instance through the use of pooled samples, point-of-care diagnosis and automation of faecal egg count (FEC) and FEC reduction (FECR) tests for assessing helminth infections and anthelmintic resistance. The use of pooled samples from sheep and cattle for herd monitoring of fluke infection has been evaluated and published. Further validation for using pooled faecal samples to assess herd level nematode infections is required.

Projects *What activities are planned or underway?*
6. Start-up for the Automation of the Mini-FLOTAC automated system

Lead Summary 12

Title:	Isolation of helminth eggs for further analysis

Research Question

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

1.The isolation of helminth stages (e.g. helminth eggs/larvae) is pivotal in order to study molecular mechanisms of anthelmintic resistance and to perform in-vitro phenotypic tests for resistance such as egg hatch assays and larval development/feeding assays
2. Isolation of helminth eggs/larvae to assess infection pressures, environmental contamination and changes in parasite epidemiology eg. due to climate change.

Challenge(s)

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

Need of rapid systems for egg/larvae isolation from fecal and environmental samples which provide reliable and representative data on eg. environmental contamination, anthelmintic resistance.

Solution Routes

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

1.Develop quick and reliable methods for helminth egg/larvae recovery and isolation
2.Develop a specific and accurate method for larvae detection on the pasture
3. Develop accurate , sensitive, practical and scalable methods for environmental (eg, soil and pasture) sampling.
4. Develop, improve and validate new phenotypic assays on harvested parasite stages to determine drug sensitivity and diagnose anthelmintic resistance eg. Egg hatching, larval development , larval motility assays .

Dependencies

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

State of the Art

Existing knowledge including successes and failures

1.Current methods used for the isolation of parasitic stages (e.g. helminth eggs and larvae) for further analysis (e.g. in-vitro assays and molecular tests) are time consuming and do not guarantee full recovery of eggs/larvae

2. Methods for environmental sampling not well developed or validated.

Projects *What activities are planned or underway?*

Lead Summary 13

Title:	Sample collection, preservation, transport and preparation of diagnostic samples for FEC/FECRT

Research Question

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

1.Determine how pooling of samples and variation in collection, storage, preservation, preparation (e.g. homogenization) affect diagnostic test outcomes?
2. Improve speed, reduce labour and improve sensitivity of sample collection.
3. Ensure negative effect of storage and transport on diagnostic outcome is avoided or at least known.

Challenge(s)

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

1.FEC and FECRT results are prone to issues such as operator dependency, method variability, and influence of sample collection, preservation, transport, preparation including all the steps of the pre-analytic phases.
2. Different parasite species have different optimal storage conditions which can be a problem samples with multiple parasite species. (eg. Lungworm larvae more vulnerable to cooling than GI nematode larvae.
3. more understanding required about the diurnal variation in egg output, influence of time of sampling and repeatability of counts.

Solution Routes

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

1.Develop open-access material, including visual tutorials demonstrating different operational procedures for sample collection, preservation, transport and preparation of samples including technical advices (collection of the samples, preservation of the samples, reagent setup, flotation solutions), critical steps, troubleshooting advices, and interpretation of results).
2.Develop eco-friendly devices with vacuum-system to preserve and transport faecal samples
3. Develop an app for geographic coding for sample identification and tracing.
4.Develop innovative tracking systems that provides a traceability of biological samples.
5. Develop a parasite group-specific set of recommendations for sample collection and storage (eg WAAVP guidelines)

Dependencies *What else needs to be done before we can solve this need?*
Promote the best practice of collection, preservation and analysis of faecal samples for the diagnosis of helminths and assessment of AR through the use of standardized procedures

State of the Art *Existing knowledge including successes and failures*
A key issue that is often ignored is the accurate collection, identification and appropriate preservation of faecal samples at the point-of-collection, which impedes their transport under varying field conditions to centralized diagnostic laboratories.

Projects *What activities are planned or underway?*
EU-MSCA-ITN application on "Systemic Anthelmintic Resistance Research (SARI) under review /