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

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

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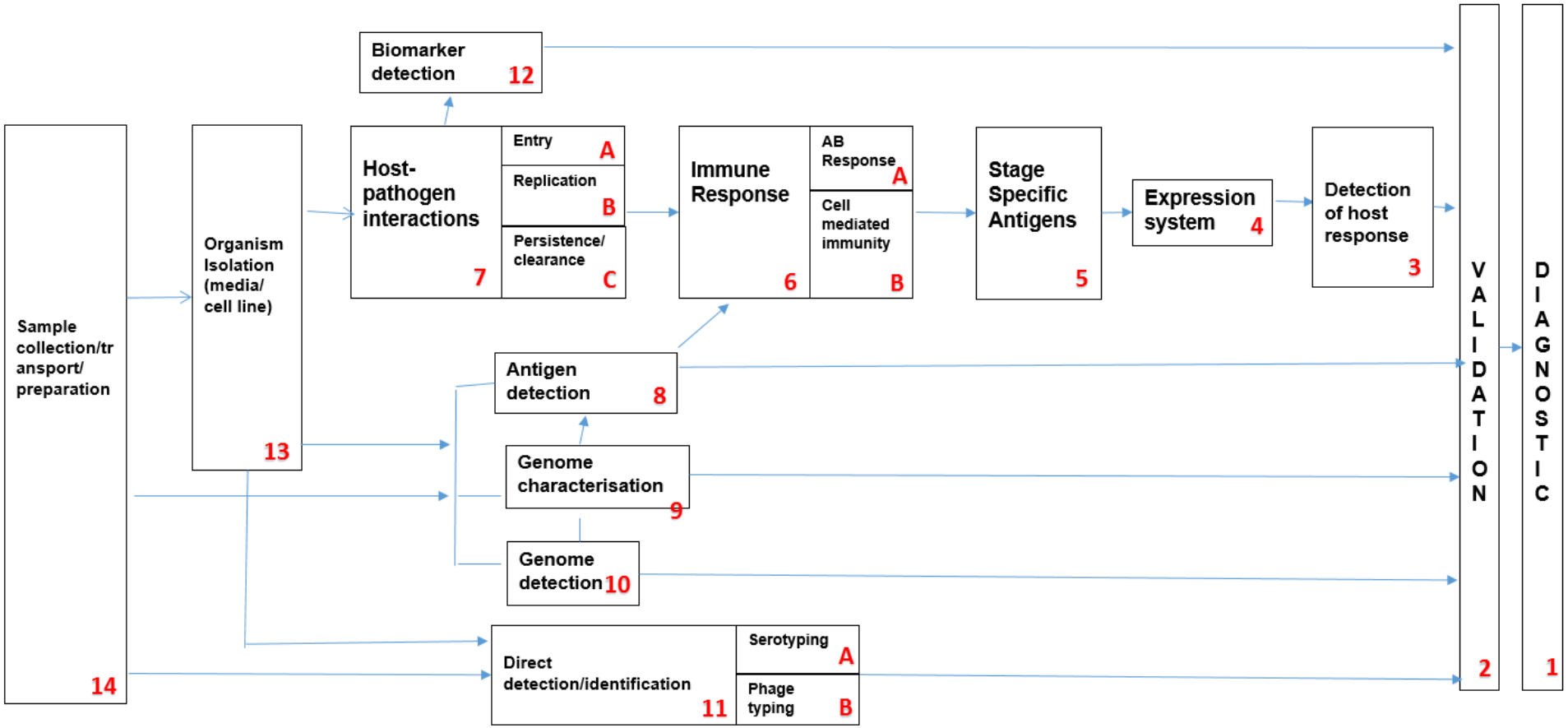
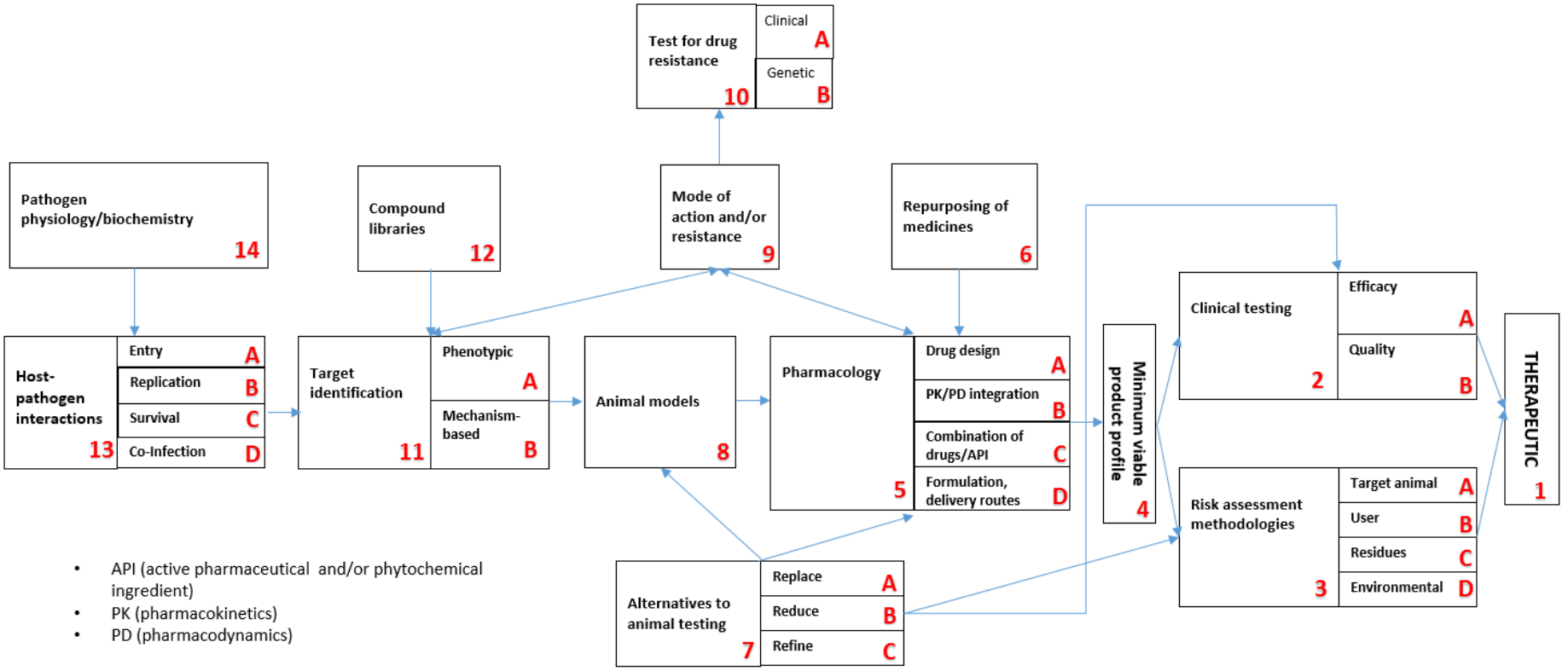
1a) Roadmap for development of diagnostic tests for helminths



1b) Roadmap for development of therapeutics for helminths



The roadmap for Therapeutics has been developed by the Livestock Helminth Research Alliance (LiHRA; June 2019) with major contributions of Carlos Lanusse, Candela Canton, Luis Alvarez, Ray Kaplan and Jozef Vercruysse.

Lead Summary1

Title:	Improved therapeutic response against helminth parasites in ruminants

Research Question

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

-*Search for approaches to achieve improved therapeutic response for* existing anthelmintics in livestock animalsin situations where their use is still valid (i.e. some drug susceptible cattle nematodes).

-Identification of novel active pharmaceutical and/or phytochemicalingredients (API) with alternative mode of action and/or expanded therapeutic response against helminth parasites resistant to other available anthelmintic drugs.

Challenge(s)

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

-Considering the increasing concern regarding the development of drug resistance, the use of pharmacology-based information is critical to design successful strategies for future helminth parasite control in livestock.

-Integrated pharmacokinetic/pharmacodynamic and clinical pharmaco-parasitology knowledge are required to identify, characterize and preserve novel anthelmintic compounds.

-To implement phenotypic and/or mechanisms-based target identification programs to come up with novel API with alternative mode of anthelmintic action
-To extend the lifespan (preserve) of the novel API with alternative modes of action
- To provide alternative parasite control measures (i.e. bioactive phytochemicals, vaccines, etc) that can be jointly/complementary used with novel API in the field in the short time.

Solution Routes

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

-A sustained and robust pipeline of new anthelmintic active compounds is critical to preserve the efficiency of intensive livestock production.
-Large scale screening technologies to identify novel chemicals active against R nematodes
-Genomic-assisted anthelmintic drug discovery programs
-Integrated basic and applied pharmaco-parasitological research with reasonable funding support.

Dependencies

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

- Improved molecular understanding of the mechanisms of drug resistance in helminth parasites.
- Improved diagnosis with genetic markers of drug resistance available for field use.
- Further interaction among academia, pharmaceutical industry and regulatory bodies.
- Greater knowledge of parasite genomics and transcriptonomics in order to identify new drug targets
- Greater knowledge of critical parasite-specific biochemical pathways that can be targeted with drugs

State of the Art

Existing knowledge including successes and failures

- Different pharmacokinetic-based approaches to enhance parasite exposure (pharmacokinetic optimisation) and the use of a mixture of molecules from different chemical families (drug combinations) have been assessed as valid strategies to control resistant parasites and to slow the selection for further resistance. These experimentally successful strategies need to be further validated in field conditions.

Projects

What activities are planned or underway?

- Valid strategies to improve parasite control in livestock animals (enhanced drug exposure, drug combinations, bioactive natural products) have been identified. Further experimental work is underway to confirm if the observed therapeutic benefits can be transferred into sustainable parasite control under field conditions. This information will be critical to define a “rational use” for any novel API introduced into future helminth control programs.

Lead Summary2 AB, 3ABCD and 4

Title:	Clinical efficacy, quality and safety assessments for a novel API with anthelmintic activity

Research Question

- What are we trying to achieve and why? What is the problem we are trying to solve?
- To develop a minimum acceptable target product profile (TPP) for a novel active pharmaceutical/phytochemical ingredient (API) with anthelmintic activity

Challenge(s)

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

- A TPP is a strategic planning tool widely used across therapeutic areas, as a guide in research and development during the search for a new API. The TPP is useful to identify critical attributes of a potential novel API before its development begins, to ensure that the final product is adapted and responds to the needs of the end-users. The most important challenge here is to elaborate a minimum viable TPP useful to guide the development of a novel therapeutic tool in parasite control. The TPP should include the following desirable/ideal promotional claims for a new API with anthelmintic properties:

- Novel chemical class. New mode of action
- Broad spectrum of anthelmintic activity (nematodes, trematodes, cestodes)
- Activity against nematodes (adult and larval stages) R to existing anthelmintic molecules
- Well tolerated in all animal species. Low tissues residues. Minimum milk and meat withdrawal times required
- Easy to be formulated for administration by different routes
- Compatible with other drug medications. Potential to be used in combination with other ATH compounds
- Adequate quality at an affordable cost in different worldwide livestock production systems
- User and environmental-friendly

Solution Routes

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

- The elaboration of a TPP to follow the efficacy, quality and safety assessments for a novel API with anthelmintic activity is a main initial challenge to be achieved. The “Attributes” (as listed in attached page below) may be achieved to guide the assessment of the clinical efficacy and safety in the process of development of any potentially new compound with improved anthelmintic activity.

- **See the proposed Target Product Profile (TPP) in attached page.**

Dependencies

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

- Further epidemiological, genetic and pharmaco-parasitological based research to understand the mechanisms of resistance in different helminth parasites of economic relevance in livestock animals. This is particularly relevant for cattle nematodes, where we still have some efficacious tools within the existing anthelmintic drugs and/or their combined use to expand the spectrum/efficacy.

State of the Art

Existing knowledge including successes and failures

- The TPP needs to define the properties of a new molecule (synthetic or natural phytochemical) to make it economically viable (worth developing). A reasonable body of information and knowledge on different strategies to improve parasite control in livestock animals under situations of anthelmintic resistance is available and it may contribute with any new development process. The accumulated knowledge for existing/traditional anthelmintic molecules should be a relevant guide in the process of development of a new API to be used in anthelmintic therapy in livestock animals.

Projects

What activities are planned or underway?

- Further experimental work is underway to confirm if the observed therapeutic benefits (drug combinations, use of phytochemicals, etc) can be transferred into sustainable parasite control under field conditions. This information will be critical to define a “rationale use” for any novel API introduced into future helminth control programs.

TARGET PRODUCT PROFILE (TPP)

Attribute	Optimum (ideal)	Minimum (acceptable)
Target animal species	All animal species	Small ruminants/Cattle
Geographic distribution	Worldwide (livestock, companion animals)	Main sheep/goats/cattle productive regions
Efficacy/Spectrum	Broad ATH spectrum (larval/adult stages of nematodes, trematodes, cestodes).	Narrow nematodicidal (adults) spectrum with activity against R nematodes
Main API quality attributes	Accommodates a wide range of doses. Optimal physicochemical properties	Restricted dose range, limited water solubility, etc
Mechanism of ATH action	Novel/differential mode of action. Activity nematodes/trematodes/cestodes.	Novel mode of action. Activity against nematodes R to BZD/LVM /ML
Route of administration	Oral/parenteral/topical in all species	Restricted to oral/intraruminal in sheep/goats/cattle

Formulations	Great formulation flexibility. Easy to administer by different routes in all animal species	Stable formulation limited to oral administration in ruminants
Pharmacokinetic properties	Excellent GI absorption, extensive tissue distribution, Large systemic exposure	Acceptable absorption pattern/systemic exposure. Good drug concentrations at the GI lumen.
Manufacturing/Cost	Accessible development/manufacturing capability in low-middle-income countries. Adequate quality at low cost.	Low technology required. Easy to manufacture in resource-limited settings at an affordable cost.
Heat stability/Shelf life	Suitable for all climatic zones. Long shelf life. No cold chain required. No special transport/storage handling requirements	Acceptable chemical stability. Easy to transport and store under controlled conditions (cold chain)
Safety/Tolerability	Large safety margin. Large therapeutic index in all animal species. Free of adverse reactions.	Safe enough to be used orally in ruminants
User safety	Fully safe for users under a variety of conditions	Non-toxic for users. Use requires some safety measures/precautions
Contraindications	None (in any animal species)	Some CI acceptable (i.e animal age, pregnancy, etc)
Interactions	None. Fully compatible with any other concomitant drug treatment	Compatible with concomitant administration of other ATH or phytochemical compounds

Tissue residues pattern	Zero withdrawal time for meat and milk	Acceptable WTs before meat/milk from treated animals is acceptable for human consumption
Environmental safety	Fully environmental-friendly formulations	Some measures should be taken to decrease the risk of adverse environmental impact

Lead Summary 5A

Title:	Pharmacological basis to design a novel anthelmintic molecule

Research Question

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

- Genomic-assisted drug discovery and/or other screening-based technologies should shortly come up with some novel molecules active against multi-resistant helminth parasites.
- *Is it possible to design a new anthelmintic molecule based on the knowledge of a biomolecular drug target?*

Challenge(s)

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

- Further knowledge on basic pharmacodynamic aspects related to anthelmintic activity is needed. The response to the following questions are the main scientific challenges to design novel anthelmintic molecules:
- Are there new/alternative potential drug binding sites in traditional (well known) molecular targets?
- Can we find potential drug binding sites in other new biomolecular binding targets?
- Which are the ´pharmacophores´ (molecular features that are necessary for molecular recognition of a ligand by a biological macromolecule)of each anthelmintic moleculeat their specific receptors?

Solution Routes

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

- Determine the three-dimensional structure of biomolecular helminth parasite targets.
- Determine the potential binding sites of those biomolecular targets.
- Determine the pharmacophoric points on each biomolecular helminth parasite target.
- Search for new potential anthelmintic drugs using computer-based methods of drug design.

Dependencies

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

- A clear identification of the true biomolecular target/s (the target/s responsible for drug effect) for each anthelmintic (traditional and novel compounds).

State of the Art

Existing knowledge including successes and failures

- Current on-going work is focussed on the understanding of the pharmacodynamic basis of anthelmintic action for most of the available anthelmintic molecules.

Projects

What activities are planned or underway?

- Ongoing genomic-assisted drug discovery and/or other screening-based technologies should shortly come up with some novel molecules active against multiple-resistant parasites.

Lead Summary 5B

Title:	Pharmacology: PK/PD integration

Research Question

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

- Integration between the pharmacodynamic (drug-receptor interaction) and pharmacokinetic (time course of changes on drug concentration) data is critical to predict and optimise anthelmintic activity. The response to the following questions should drive the pharmacology-based search for new molecules:
- Is it possible to predict drug efficacy (PD) based on PK data?
- Is drug activity (efficacy) directly dependent on drug concentration achieved at the site of parasite location?
- Are plasma/serum drug concentrations a good indicator to predict either drug concentration at the site of parasite location or amount of active drug accumulated within a target helminth?
- How do plasma/serum drug concentrations correlate with drug levels attained at different target sites (i.e. GI fluids, skin, lung, liver, etc.) for each drug type (i.e. benzimidazoles, macrocyclic lactones, etc.)?
- How could the PK changes (induced by host physiology, co-administered drugs, etc.) affect the anthelmintic therapeutic response?
- Which are the main routes of drug entry into target parasites (oral, transcuticular/tegumental)? This information in needed for each anthelmintic/chemical group at each target parasite.
- Are there are other reliable parameters (in addition to the number of collected parasites/number of eggs passed in faecal material) to evaluate drug effect against helminths? (i.e. tegumental/cuticular alterations, loss of motility, etc.)

Challenge(s)

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

- To determine drug concentration required to kill/remove parasites. This information is necessary for each anthelmintic/chemical group, in different target parasites and in the different animal species in which the drug is intended to be used.
- To develop analytical techniques to accurately quantify drugs and/or metabolites in different tissues/fluids, including parasite material.
- To identify a reliable parameter to estimate when a parasite has been irreversibly affected by an anthelmintic molecule.

Solution Routes

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

- Design and execution of experiments in order to collect PK (in plasma and organic fluids/tissues) and efficacy data.
- Development of in vitro/ex vivo approaches in order to find reliable parameters (surrogates) to estimate when the drug has irreversibly affected a parasite.

Dependencies

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

- The `minimum effective concentration´ for anthelmintic drugs needs to be determined (for different drugs/metabolites, in different fluids/tissues, different animal species and for different parasites). Basic and detailed PK data for some existing anthelmintic drugs and for novel ones is needed (in some animal species). The development of accurate PK/PD models for use with anthelmintic drugs is urgently required.

State of the Art

Existing knowledge including successes and failures

- The available integrated pharmacokinetic/pharmacodynamic and clinical pharmaco-parasitology knowledge is useful to optimize the use and to preserve traditional/novel anthelmintic compounds. The available basic pharmaco-parasitological information for different traditional anthelmintics (in different animal species) is relevant to improvinganti-parasitic therapy.

Projects

What activities are planned or underway?

- Integrated pharmacokinetic/pharmacodynamic and clinical pharmacology knowledge is required to preserve both long-time existing and more modern anthelmintics. The development of research projects focussed on the simultaneous and integrated characterization of PK and PD (efficacy) data for novel anthelmintic molecules must be a high priority in the field.

Lead Summary 5C

Title:	Pharmacology: Combination of drugs/API

Research Question

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

- The rationale behind using drug combinations is based on the fact that individual worms may have a lower degree of resistance to a multiple component formulation (each chemical with different mode of action/resistance) compared to that observed when a single anthelmintic molecule is used. The resulting low number of surviving parasites with resistant genotypes would be diluted into the nematode population in refugia, and the resistant worms would take longer time to increase to the point of being predominant. The challenge will be to identify a combination between drugs (or between a synthetic drug and a phytochemical ingredient) resulting in a broad spectrum of very high activity against resistant helminth parasites. Response to the following questions should focus the research in this specific pharmacology-based field:
- Could anthelmintic combinations increase drug efficacy if AR is already present?
- Could anthelmintic combinations delay the development of resistance?
- Could the use of anthelmintic combinations be a sustainable control measure in field conditions?
- Are there associated risks to the use of anthelmintic combinations? Host toxicity? Tissue residues?
- Should anthelmintic combinations be used as fix preparations or as separate compounds concomitantly used?
- How can the favorable anthelmintic synergistic effects of drug combinations be optimised at the farm level?
- Which will be a rationale rotation scheme to extend the lifespan of those advantageous drug mixtures?
- What is the level of efficacy that should be targeted in a combination to maximize/optimize the ability to delay the development of resistance.

Challenge(s)

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

- Lack of plasma PK data for different anthelmintics administered alone or in combination in some animal species (i.e. goats, sheep).
- Lack of efficacy data for different anthelmintics administered alone or in combination in different animal species.
- Lack of plasma PK and efficacy data of anthelmintics administered alone or combined with different API (i.e. metabolic inhibitors, transporters modulators/substrates, etc.).

- Further understanding of underlying mechanisms behind anthelmintic resistance.
- Long-term field trials testing the strategy of using combinations to delay the development of AR

Solution Routes

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

- PK studies of different traditional/novel API with anthelmintic activity administered alone or in combination.
- Efficacy studies for different anthelmintics administered alone or in combination.
- Drug tissue residues depletion for molecules administered alone or in combination.
- ´Target´ species toxicity studies of different anthelmintics administered alone or in combination.
- Long-term studies investigating the benefits of combinations in delaying the development of AR

Dependencies

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

- Development of novel anthelmintic molecules. Search of new APIs useful to be used in combination. This is a critical issue affecting all the Lead Summary 5C.

State of the Art

Existing knowledge including successes and failures

- Plasma PK data of different anthelmintics administered alone or in combination in different animal species is available.
- Efficacy data of different anthelmintics administered alone or in combination in different animal species is also available.
- We also have available data on plasma PK data for some anthelmintics administered alone or combined with different API available (i.e. benzimidazoles with metabolic inhibitors, endectocides with P-gp modulators/substrates, etc.).

Projects

What activities are planned or underway?

- The occurrence of potential pharmacokinetic and/or pharmacodynamic interactions between drug components highlight the need of deeper pharmacological-based research to identify the advantages/disadvantages of the use of combined drug preparations for

anthelmintic control in livestock. This work is currently undergoing for some of the traditional existing anthelmintic molecules used in combination in cattle.

Lead Summary 5D

Title:	Pharmacology: Formulation, delivery routes

Research Question

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

- Any potential new development of an API (synthetic drug or phytochemical) with anthelmintic activity needs to be prepared as a pharmacotechnical formulation compatible for use in the target animal species. The following research questions should be addressed:
- What is the best formulation (concentration, solution/ suspension, pH, stability, etc.) for each novel identified anthelmintic molecule?
- What is the best route of administration to achieve the best pharmacological (systemic exposure) and clinical (efficacy) response in each target animal species?

Challenge(s)

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

- Improved absorption of existing/novel drugs included in pour on formulation intended for a systemic anthelmintic effect.
- Good acceptability of oral formulations in ruminants.
- Development of a device for parenteral (subcutaneous, intramuscular) administration with automated delivery, etc.

Solution Routes

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

- To undertake plasma PK, tissue residue depletion and clinical efficacy studies for the new formulations containing novel active ingredients. Target species toxicity studies should be also performed.

Dependencies

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

- Development of new drug formulations.
- Development of new vehicles and/or excipients.
- Development of new technologies for anthelmintic administration in farm animals

State of the Art *Existing knowledge including successes and failures*
- New formulations are available or under current experimentation (i.e. nanoencapsulated drugs, water soluble formulations of benzimidazole anthelmintics, etc.). PK studies involving new available formulationsare undergoing. - Efficacy studies in different animals models involving new formulations are available. All this knowledge may be relevant to be applied in the pharmacotechnical development of an improved formulation to deliver a “novel API” with anthelmintic activity.

Projects *What activities are planned or underway?*
- See comments in “State of the Art”

Lead Summary [6]

Title:	Drug Repurposing

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*
- Drug repurposing has acquired special relevance in several therapeutic fields. Could the use of old drugs for new indications be a valid strategy to be explored in resistant nematodes? The following specific questions should be used as a guide to assess the search for drug repurposing: - Is it possible to find new anthelmintic indications for old available drugs? - Could these potential new indications improve helminth/parasite control in practice? - Could these potential new indications improve helminth/parasite control in practice in the context of anthelmintic resistance? - Repurposing old medicines may include old drugs not originally intended for parasite control. Could they be used alone or in combination with existing anthelmintics? - Can current drugs be used at higher doses, perhaps in combination to elicit other loci of activity not normally activated at normal therapeutic levels?

Challenge(s)

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

- Gathering information on new anthelmintic indications for old existing drugs.
- Further understanding of underlying mechanisms behind anthelmintic resistance.
- Efficiently screening libraries of old drugs against multiple species of parasites. (see Lead Summary 12)

Solution Routes

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

- Efficacy studies using old drugs with "potential anthelmintic activity" administered at different doses, co-administered, and administered against different stages of different helminth parasites.

Dependencies

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

- Validated animal models involving different target and/or model parasites.

State of the Art

Existing knowledge including successes and failures

- High oxfendazole doses (30 mg/kg) demonstrated a high efficacy against *Taenia solium* cysticerci in pigs, and against adult liver flukes in pigs and sheep.
- High doses (x 10) of albendazole and ivermectin ´reverse´ resistance in *H. contortus*. Some of the available knowledge is encouraging regarding the potential of old existing drug (used under novel/alternative indications) to control helminth parasites.

Projects

What activities are planned or underway?

- Further pharmaco-parasitological work to explore the potential of oxfendazole and flubendazole under new/alternative indications for parasite control in livestock are in progress.

Lead Summary [7a]

Title:	Alternative to Animal Testing - Replace

Research Question

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

- There are increasing societal pressures to reduce and replace the use of animals in research
- Reducing and/or replacing the use of animals in research will require the development of alternative approaches that can replicate the complex host and the host-parasite dynamics that defines the pharmacokinetics and pharmacodynamics of a drug when administered to a animal.
- *Ex vivo* and *in vitro* alternatives to the use of animals needs to provide equivalent, or at least translatable data for efficacy, safety, etc.

Challenge(s)

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

- The adult stages and many of the larval stages of helminth parasites live inside of animals, and cannot survive and develop outside of the animal host
- For the most part, we lack the knowledge necessary to replicate the host milieu in an *ex vivo* or *in vitro* environment
- Advances in the methods and procedures for the *ex vivo* or *in vitro* culturing of parasitic stages of helminths are needed

Solution Routes

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

- Studies investigating novel approaches for replicating the host environment in an *ex vivo* or *in vitro* system are needed
- Improved knowledge of host-parasite dynamic can enable the development and use of *in silico* experiments, that can be used to test relevant aspects of multiple different dosing regimes

Dependencies

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

- Additional knowledge of helminth physiology and on the role of host proteins, miRNAs, lectins, and other host signals in creating an environment conducive to the parasite's survival
- In addition to knowledge of general mechanisms, host-parasite-specific mechanisms will also need to be understood.

State of the Art *Existing knowledge including successes and failures*
- Several protocols for culturing parasitic stages of helminths exist; however, they only allow for short-term survival - It is still not possible to successfully complete the life cycle of a helminth parasite outside of a host animal.
Projects *What activities are planned or underway?*
- Gathering of additional basic understanding of the host-parasite interaction. - Investigations into methods and techniques to improve the ability to culture parasite stages in an in vitro system

Lead Summary [7b, 7c]

Title:	Alternative to Animal Testing – Reduce, Refine

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*
- Replacing animals in helminth research will be quite difficult in the foreseeable future do the current state of knowledge and technologies. Consequently, approaches that can refine and reduce the use of animals is a more achievable goal in the near-term - Replacing higher mammals with rodents will potentially both reduce and refine since rodents are highly inbred and thus there is less to animal-to-animal variability. High variability in animal testing is a problem because it demands the use of larger group sizes and therefore more animals.

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
- Most helminth parasites are quite host-specific, making it difficult to develop rodent models for those parasites - Rodent model systems will need to be developed and validated independently for each helminth species - Development of standardized molecular markers that can differentiate between different strains of the same parasite species

- Make use of state-of-the-art statistical models that can increase the power of experiments to draw relevant biological conclusions using fewer animals

Solution Routes

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

- (Refine) Develop rodent models that can replace the use of higher mammals (e.g. dogs, cats, horses, ruminants)
- (Reduce and refine) Test multiple strains (such as drug-susceptible and drug-resistant) and species of parasites in a single animal and use molecular markers to determine species-specific and strain-specific efficacy.
- (Reduce) Use statistical models to improve the information that can be obtained from experiments using fewer animals

Dependencies

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

- Research into modifying and adapting important species of helminths to survive and develop in a rodent system. This type of research has had minimal funding in the past few decades.
- Genetics and genomics research to enable the development of reliable and biologically relevant molecular markers for species, strains within a species and resistance status for each important drug class.

State of the Art

Existing knowledge including successes and failures

- A gerbil and a rat model exists for testing drugs against *Haemonchus contortus* and *Trichostrongylus* spp.
- Diagnostic panels of microsatellite markers have been developed for several important helminth species; these can be used to differentiate between strains of the same species
- Molecular assays exist to detect and measure resistance to benzimidazole anthelmintics

Projects

What activities are planned or underway?

- Genomics and genetic research is on-going to identify the genetic basis of resistance to multiple anthelmintic classes

Lead Summary [8]

Title:	Animal Models

Research Question

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

- Develop rodent models to replace the use of higher mammals (e.g. cats, dogs, horses, ruminants)
- Rodents are highly inbred and therefore there is less animal-to-animalvariability.
- Develop animal models that do not use mammals (e.g. fish)

Challenge(s)

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

- Most helminth parasites are quite host-specific, making it difficult to develop animal models for those parasites
- Model systems will need to be developed and validated independently for each helminth species
- Model systems are unlikely to be translatable for toxicity studies because there are many host-specific toxicities that are not seen in other animals

Solution Routes

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

- Perform research to test the potential of multiple different rodent and non-rodent model systems to support the development of multiple important parasite species of domestic animals (e.g. cats, dogs, horses, ruminants)
- Testing and validating non-traditional screening systems such as a zebrafish nematode model

Dependencies

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

- No real obstacles to performing this line of research other than funding

State of the Art

Existing knowledge including successes and failures

- A gerbil and a rat model exists for testing drugs against *Haemonchus contortus* and *Trichostrongylus* spp.

Projects *What activities are planned or underway?*
- Difficult to know - An in vivozebrafish nematode model is being examined by investigators at Oregon State University Veterinary College

Lead Summary 9

Title: Mode of action/Resistance

See text in Lead Summaries 1, 5 and 14.

Lead Summary 10 A, B

Title:	Detection of drug resistance: clinical and genetic tests

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*
- Can we achieve standardized guidelines for clinical and genetic tests to detect drug resistance? We needofficial guidelines across a variety of animal and important parasite species. - Can we have a test for field use to detect early stages of drug resistance?

- Could it be possible to validate more genetic markers as predictive of drug resistance under field conditions?

Challenge(s)

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

- Lack of standardization of the faecal egg count reduction test(FECRT) to detect anthelmintic resistance, especially in cattle (is the presence of drug resistance indicated by a FECR of less than 90 or 95%? Is it essential to have a control group?; Is it performed with individual or composite faecal samples?; etc.).
- Lack of accessible tests for early detection of anthelmintic resistance available to use in the field.
- Poor understanding of suitable genetic markers of resistance for the available anthelmintics, including the most commonly used such as the macrocyclic lactones.

Solution Routes

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

- Implement practical on-farm tests with high sensitivity for detection of anthelmintic resistance in livestock animals.
- Implement coherent and clear guidelines for different methods to detect resistance to anthelmintic compounds.
- Implement genomic approaches to characterize resistance, in order to identify genetic markers for the diagnostic of anthelmintic resistance.

Dependencies

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

- Improve knowledge about the genomes of livestock helminth species.
- Review current guidelines about clinical tests for drug resistance (as FECRT): what are the best methods for calculating confidence intervals? ; what is the sample size of animals and the number of eggs pre-treatment that would allow a reliable assessment of drug efficacy (provide high statistical power)?

- Specialized equipment for performing genetic tests (for example, pyrosequencers or MiSeq DNA sequencers) should be more widely available in veterinary laboratories.

State of the Art

Existing knowledge including successes and failures

- The traditional method for detecting drug resistance on farms, the faecal egg count reduction test, presents variable results, depending on study design, parasite/hosts interactions, egg counts, etc. Additionally, the resistance status of the parasites is not known until after an anthelmintic treatment.
- Although the FECRT is the only test available at the farm level, it has poor sensitivity and cannot reliably detect resistance in the early emerging stages, whereas genetic tests (pyrosequencing, Illumina sequenicng) can detect resistance parasites on a farm before a decrease in drug efficacy is detected.
- Genetic tests, if readily available, would be useful to veterinarians and producers because of the ability to detect resistance before treatment.
- Genetic tests allow an estimation of the percentage of resistant parasites in the parasite population in a herd/flock (for example through the analysis of SNPs 167, 198 and 200 for the detection of benzimidazole resistance).

Projects

What activities are planned or underway?

- Standardized guidelines for performing and interpreting results of the FECRT to detect drug resistance are in preparation.
- Standardized guidelines for genetic tests to detect drug resistance are in preparation.
- Different field-based methods for nematode faecal egg count diagnostics are under evaluation.
- Expand genetic tests to other parasite speciesand other anthelmintic classes (currently, *H. contortus* resistant to benzimidazoles isthe most studied parasite species).

Lead Summary [11]

Title:	Target Identification

Research Question

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

- Identify new protein targets for anthelmintic drug development

Challenge(s)

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

- Knowledge of the genomics, transcriptomics, and general physiology of helminths is still rather poor; 40% of more of nematode genes have unknown function
- We still lack knowledge of the full spectrum of targets of currently used anthelmintics; evidence suggests these drugs may be targeting more loci than is currently known.
- If current drugs are highly effective because they target multiple loci in worms, then identifying individual targets may turn out to be a failed approach. It is noteworthy that all anthelmintics to date were discovered using organismaltesting and no target-based drugs have yet been developed despite more than two decades of effort.

Solution Routes

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

- Improve knowledge of how current anthelmintics work by identifying all loci where they have activity and/or where changes in specific loci cause resistance to the drug
- Improve knowledge of the genomics, transcriptomics, and general physiology of helminths
- Appreciate that single target drugs may fail to be sufficiently effective by themselves, but might be effective in a cocktail of several drugs each targeting a different loci.

Dependencies

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

- Currently available technologies should be sufficient to solve this need. The need will be for sufficient funding to support the necessary research efforts.
- Strong target/compound leads will be pursued by individual companies who will not want to share their intellectual property. However, those leads may fail as independent targets/compounds. Thus a system or consortium should be put into place where multiple leads from several companies that fail as a stand-alone product can be investigated together for the potential to develop a cocktail of compounds that may yield an effective product.

State of the Art

Existing knowledge including successes and failures

- Despite more than two decades of research, no anthelmintics using a targeted approach have been developed.
- This failure should lead to questions about the validity of this approach, however, as knowledge and tools continue to improve, success is still possible. Additionally, a cocktail of compounds discovered using a target-based approach might be highly effective.

Projects

What activities are planned or underway?

- Multiple private and public institutions throughout he world are pursuing research into discovering new active targets for new anthelmintics

Lead Summary [12]

Title:	Compound Libraries

Research Question

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

- High throughput screening of existing compound libraries to identify candidate compounds with anthelmintic activity

Challenge(s)

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

- Having robust and relevant parasite screening assays with a high probability of identifying potential lead compounds
- Depending of the mechanism of action of a given compound, different types of assays will be more or less likely to discover potential lead compounds
- Motility assays are frequently used for screening compounds for anthelmintic activity because this is a phenotype that is easily measured in a high throughput format. However, many compounds may not demonstrate activity with short-term motility assays.
- Some drugs may require a competent immune system for activity, and these will likely not yield a positive hit in an *in vitro* assay; for example ivermectin does not have significant *in vitro* activity against filarial nematodes but is highly effective *in vivo*. Similarly, motility-based assays fail to reliably detect resistance to ivermectin (and other macrocyclic lactone anthelmintics) across multiple species
- All current *in vitro* assays used for screening compounds utilize non-parasitic (free-living) stages, which are not the stages in the animal that are targeted by the drug. Thus, some compounds may not kill the stage of parasite used in the assays even though it might have activity against the parasitic relevant stage. Such compounds will thus be overlooked. Consequently, developing *in vitro* or *ex vivo* culture systems that can maintain adult nematodes for extended periods of time could facilitate their use as a target in screening assays.
- Non drug phytochemical compounds with alternative mode of action may not demonstrate activity using traditional screening assays. Many of these types of compounds require long-term *in vivo* exposure to demonstrate activity, and many *in vitro* assays may not proceed long enough to demonstrate the activity.

Solution Routes

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

- Multiple phenotypes in addition to motility assays should be utilized, such as larval development assays
- For filarial worms, *in vivo* screening may be necessary. To minimize the number of *in vivo* tests, compound hits using other assays could be tested against filarial worms in an *invitro* assay and if the activity is significantly less than in the

original screen then the compound could be tested against filarial worms in an *in vivo* model.
- For phytochemical compounds, new novel in vitro assays may need to be developed that can better replicate the ruminant digestive system.

Dependencies

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

- Presently high throughput assays are expensive to set up; consequently a large investment is required in both equipment and personnel. This restricts such screening to large pharmaceutical companies and large centralized laboratories with significant funding. Consequently, developing less expensive technologies could facilitate a more decentralized system, and therefore the screening of more compounds in a variety of assay systems.
- Making compound libraries more easily available for laboratories to access
- Having a centralized publically accessible database system will facilitate compound screening and anthelmintic discovery by smaller more specialized laboratories.

State of the Art

Existing knowledge including successes and failures

- New image analysis software has made the development of inexpensive systems achievable and several are now available and practical for smaller scale laboratories.

Projects

What activities are planned or underway?

- Multiple private and public institutions throughout he world are screening compound libraries to identify potential new anthelmintics

Lead Summary 13 A, B, and C

Title:	How do host and parasites interact?: Entry, survival and co-infection

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Research Question

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

- A deeper knowledge of the host-helminth parasite interaction is a critical issue for developing novel therapeutic strategies to improve parasite control in livestock. The response to the following questions should guide research in this field:
- What are the main factors that influence the host-parasite dynamic?
- Can we achieve a better understanding of host immune reactions responsible for protection against helminths in livestock animals?
- Can we further identify the parasite molecules (antigens) that induce the immune responses in the host?
- How do different parasite species interact within the host? Can one affect the survival of other parasite species?
- Does an anthelmintic treatment affect the immune response of the host?
- How do host immunity and different parasite species influence the response (efficacy) of an anthelmintic treatment?

Challenge(s)

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

- To determine parasite and host factors leading to persistent infection in the host.
- Poor understanding of parasite-host interactions at the molecular level.
- Lack of knowledge about mechanisms of immunity against parasites in livestock animals (particularly in in cattle).
- What are the factors that influence co-infection among different helminthspecies?
- To determine how the host immunity status influences the efficacy of an anthelmintictreatment.
- To what extent does the interaction with the gut microbiome effect the host-immune response to parasites and how does this impact the overall immune state of the host?

Solution Routes

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

- Investigation of the main factors that influence the host-parasite dynamic under natural field conditions.
- Field studies of host immune responses(innate and acquired) in animals exposed tonatural parasite infection (co-infection with different parasite species).
- Evaluation of the efficacy of an anthelmintic treatment in livestock animals with different immunity status under field conditions.
- Study the interactions of the 'nemabiome' and the microbiome

Dependencies

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

- Improvement of tools to measure immune responses in livestock animals.
- Better understanding of parasite-host interactions at the molecular level.
- Better description of interactions between field parasite populations through molecular tools to identify helminth species.
- Better understanding of nemabiome-microbiome interactions at the molecular level.

State of the Art

Existing knowledge including successes and failures

- Ability to maintain immune responses against helminths is different between livestock animals.
- Existing knowledge about the complexity of parasite immunity in livestock animals.
- Lack of studies under field conditions with animals naturally parasitised.

Projects

What activities are planned or underway?

- Studies to describe the immune response against helminths in different livestock animals are underway.
- Investigation of how the host immune status affects the effectiveness of anthelmintic treatment in livestock animals is planned.

Lead Summary 14

Title:	Further knowledge on parasite physiology/biochemistry

Research Question

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

- Further knowledge on different aspects of the physiology and biochemical pathways in helminth parasites is needed. The search for novel drug targets in helminths is required. Are there unidentified and critical biochemical pathways in helminths?Could these "unknown" pathways (or biomolecules involved in the pathways) be used as potential drug targets?

Challenge(s)

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

- Development of *in vitro/ex vivo* techniques useful to explore the physiological role of different biochemical pathways in parasites.
- Development of different technologies to assess the effect of drug interferences on "novel biochemical pathways" in parasites.

Solution Routes

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

- Mechanistic-based studies to understand the physiological role of different biochemical pathways in parasites.
- Genetic-based studies to understand the physiological role of different biochemical pathways in parasites.
- Evaluation of the differences in those biochemical pathways among different helminth parasites and/or in different stages of the same parasite.

Dependencies

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

- *In vitro/ex vivo* techniques useful to explore the physiological role of different biochemical pathways in parasites.
- *In vitro/ex vivo* techniques useful to explore the consequences observed after interference of different biochemical pathways in parasites.

State of the Art

Existing knowledge including successes and failures

- Considering the limited existing knowledge, a wide search for novel drug targets based on parasite physiology/biochemistry should be implemented. This is considered a critical issue to be able to identify novel anthelmintic molecules to deal with resistant helminths in the future. Some examples (among many others) are fields such as: - Characterization of serotoninergic G-protein coupled receptors in cestodes. - Alternative metabolism and energy-production mechanisms in different helminth parasites. - Characterization of aquaporins in cestodes and trematodes.
Projects *What activities are planned or underway?*
- Characterization of aquaporins in cestodes and trematodes and their role in parasite physiology and their assessment as drug targets.