STAR-IDAZ
International Research
Consortium on Animal Health

# 1. Roadmaps for the development of diagnostic tests and therapeutics for helminths

# 2. Roadmaps for the development of candidate vaccines and control strategies for liver fluke and nematodes

# 3. Roadmaps for the development of candidate vaccines, diagnostic tests and control strategies for FMD

# 4. Roadmap for research to underpin the development of control strategies for ASF

*SIRCAH Deliverable 3.4*

*30/09/2019*

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

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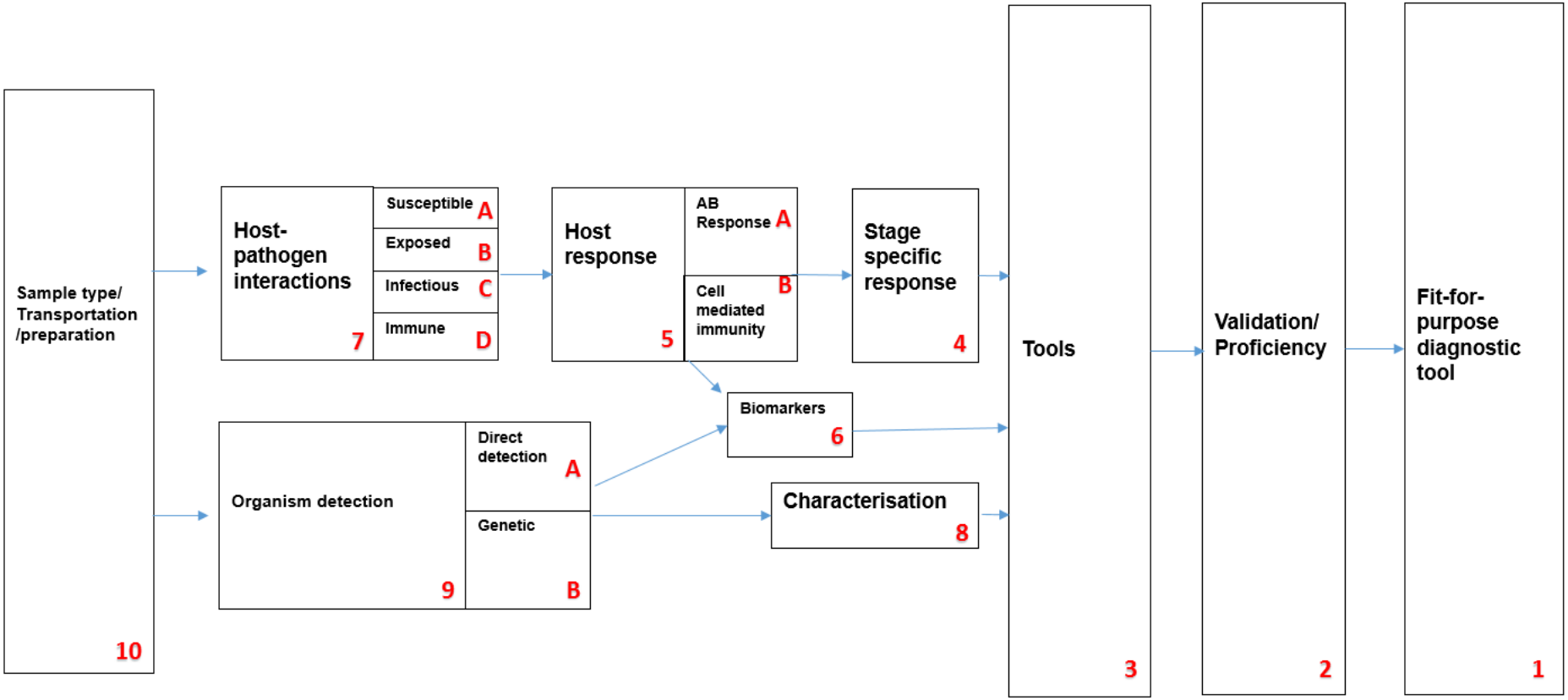
# 3b) Roadmap for the development of diagnostic tests for FMD




*FMD roadmap lead summaries are in draft form until validated by the GFRA*

# FMD Diagnostics - Lead Summary 1

| **Title:** | Development of reliable and rapid tests allowing to identify vaccinated and exposed animals, including pre-clinical, subclinical and animals |
|---|---|

**Research Question**

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

To produce new reliable and rapid diagnostic tests allowing identification of vaccinated and exposed animals, including peri-clinical, subclinical and carrier animals.

**Challenge(s)**

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

Develop test allowing for pen-side antigen detection and serotype or strain determination and antigen capture.
Develop tools for different serotypes for genetic/antigenic typing, especially vaccine matching.
Proof-of-concept testing of herd immunity test correlated with efficacy of vaccines.
DIVA.

**Solution Routes**

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

Assess the feasibility of infrared thermography as an FMD screening tool under different environmental field conditions in healthy and diseased animal populations. Assess the potential application of this technology to aid in the identification and sampling of suspected animals for confirmatory diagnostic testing.

**Dependencies**

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

Host response.
PCR/LAMP.
Antigen capture.
Validation.

**State of the Art**

*Existing knowledge including successes and failures*

Molecular and genetic technologies, including sequencing, are developing at an increasing rate both in terms of capability and affordability. The development of RT-LAMP represents an important breakthrough allowing greater use and access to molecular diagnostics. It is now possible to determine virus serotype using PCR, although only for certain virus pools, continued progress is needed to cover the global spectrum of FMD viruses. Progress has also been made in the development of pen-side rapid diagnostics, some with the ability to determine serotype. However, further advances in pen-side serotype or strain determination would benefit both FMD-free countries and endemic countries with limited access to well-resourced laboratories. There are validations for a few serotypes. Recently developed methods are not already tested in the field.

**Projects**

*What activities are planned or underway?*

# Lead Summary 2

| **Title:** | Validation of a diagnostic tests for different serotypes and in field conditions |
|---|---|

**Research Question**

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

New diagnostic methods have been developed but validation is lacking for some serotypes and in field conditions.

**Challenge(s)**

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

Serologically differentiation among cross-reacting serotypes.
Evaluate and validate LFDs/Ag detection pen-side tests for surveillance, response, and recovery.
Obtain field validation data for isothermal tests.
Validate portable equipment before being used in emergency situations.
Evaluate sensitivity and specificity of diagnostic tests and surveillance systems at global, regional, and national scale.

**Solution Routes**

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

Validate field tests in different endemic areas during disease outbreaks.
Negotiate access to archived samples.
Methods must be tested in the field.

**Dependencies**

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

Host response.
PCR/ loop-mediated isothermal amplification (LAMP).
Antigen capture.
Access to samples for performing validation tests.

**State of the Art**

*Existing knowledge including successes and failures*

Molecular and genetic technologies, including sequencing, are developing at an increasing rate both in terms of capability and affordability. The development of RT-LAMP represents an important breakthrough allowing greater use and access to molecular diagnostics. It is now possible to determine virus serotype using PCR, although only for certain virus pools, continued progress is needed to cover the global spectrum of FMD viruses. Progress has also been made in the development of pen-side rapid diagnostics, some with the ability to determine serotype. However, further advances in pen-side serotype or strain determination would benefit both FMD-free countries and endemic countries with limited access to well-resourced laboratories. There are validations for a few serotypes. Recently developed methods are not already tested in the field.

**Projects**

*What activities are planned or underway?*

# Lead Summary 3

| **Title:** | Development of expression systems for FMD diagnostic reagents |
|---|---|

**Research Question**

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

To develop expression system for FMD diagnostic reagents that would not require specialised biosafety level 3 facilities to be produced securely.

**Challenge(s)**

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

Develop simple methods for RNA extraction.
Identify new expression systems.

**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*

The use of recombinant technology as an alternative source of test antigens has shown promise in several studies.

**Projects**

*What activities are planned or underway?*

# Lead Summary 5

| **Title:** | Investigate animal response to FMDV infection to identify diagnostic targets |
|---|---|

**Research Question**

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

To identify the mechanisms operating in immune animals, establishing the role and timing of antibodies and cell mediate immune responses to FMDV infection.

**Challenge(s)**

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

Investigate the immune response in target animals.

**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?*

Improve understanding of host virus interaction.
Genome sequence of various FMDV isolates.

**State of the Art**

*Existing knowledge including successes and failures*

Recent studies have shed light on the mechanisms underlying formation of the bovine B- and T-cell response; there is also a greater understanding of the significance of non-neutralising antibodies during FMDV infection and the interactions of antibody-bound virus with immune cells.

**Projects**

*What activities are planned or underway?*

# Lead Summary 5A

| **Title:** | Identify antibodies in response to FMDV infection |
|---|---|

**Research Question**

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

To establish if the antibody response can be used early on in infection and in establishing the infectious status of the animal.

**Challenge(s)**

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

Establish if the antibody response can be used early on in infection and in establishing the infectious status of the animal.

**Solution Routes**

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

Characterisation of the antibody response following infection.

**Dependencies**

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

Host-pathogen interaction.
Antigen detection.
FMDV genome.

**State of the Art**

*Existing knowledge including successes and failures*

**Projects**

*What activities are planned or underway?*

# Lead Summary 5B

| **Title:** | Identify cell mediated immunity in response to FMDV infection |
|---|---|

**Research Question**

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

To define immunological parameters involved in immune response against homologous and heterologous infection, besides antibody titres.

**Challenge(s)**

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

Confirmation of the infection status, including the detection of infection at the earliest possible stage.

**Solution Routes**

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

Characterisation of the cellular immune responses during the different stages of infection and comparison with the antibody response.

**Dependencies**

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

Host-pathogen interaction.
FMDV genome.

**State of the Art**

*Existing knowledge including successes and failures*

**Projects**

*What activities are planned or underway?*

# Lead Summary 7

| **Title:** | Characterising Host Pathogen interaction in FMD infection |
|---|---|

**Research Question**

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

To gain an improved understanding of how FMDV enters, replicates and survives in and is released from infected cells.

**Challenge(s)**

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

Explain the mechanisms that govern permissiveness to infection of distinct species- breeds, and how do these factors apply to variability of virulence across cattle breeds.

**Solution Routes**

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

Exploitation of precise, quantitative, high-throughput molecular techniques to study FMDV for advancing understanding of pathogenesis at the cellular level.
Characterise the interactome of FMDV

**Dependencies**

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

Genome sequence of various FMDV isolates.

**State of the Art**

*Existing knowledge including successes and failures*

How the virus evades innate immunity:
FMDV has evolved many strategies exploiting each region of its small RNA genome. These include: (a) inhibition of IFN induction at the transcriptional and translational level, (b) inhibition of protein trafficking; (c) blockage of specific post-translational modifications in proteins that regulate innate immune signalling; (d) modulation of autophagy; (e) inhibition of stress granule formation; and (f) *in vivo* modulation of immune cell function.

**Projects**

*What activities are planned or underway?*

# Lead Summary 7B

| **Title:** | Development of reliable and rapid immunological tests allowing to identify exposed animals, including peri-clinical, subclinically infected and recovered animals |
|---|---|

**Research Question**

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

To develop reliable and rapid immunological tests allowing to identify peri-clinical, subclinically infected, and recovered animals.

**Challenge(s)**

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

Identify peri-clinical, subclinical and carrier animals.
Need for serological tests to show recovery (absence of circulating virus).
Develop serotype specific rRT-PCR assay(s).
Generate immunological reagents for testing wildlife.

**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?*

Immune response.

**State of the Art**

*Existing knowledge including successes and failures*

The use of recombinant technology as an alternative source of test antigens has shown promise in several studies.

**Projects**

*What activities are planned or underway?*

# Lead Summary 7C

| **Title:** | Establishing the mechanisms of virus persistence/clearance from infected cells |
|---|---|

**Research Question**

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

To establish if there are biological markers of persistence and identify them.

**Challenge(s)**

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

Identify markers of carrier state.

**Solution Routes**

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

Proteomic approaches in persistent /not persistent cells would allow to detect specific markers of persistence.

**Dependencies**

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

Understand the mechanisms of persistence.
The pathogen and host factors that contribute to FMDV persistence are currently not understood.

**State of the Art**

*Existing knowledge including successes and failures*

Some mechanisms of persistence at the cellular level (in the soft palate) begin to be understood.

**Projects**

*What activities are planned or underway?*

# Lead Summary 7D

| **Title:** | Development of reliable and rapid immunological tests allowing to identify vaccinated animals |
|---|---|

**Research Question**

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

To develop reliable and rapid immunological tests allowing to identify vaccinated animals.

**Challenge(s)**

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

Level, quality and standardisation of level of Abs for identification of vaccinated animals.
Investigate seroconversion of vaccinated cattle.
Interference of maternal antibody with DIVA tests.
Investigate relationship between the degree of vaccine purification and DIVA test performance.

**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?*

Immune response.
Identify DIVA targets.

**State of the Art**

*Existing knowledge including successes and failures*

The use of recombinant technology as an alternative source of test antigens has shown promise in several studies. Several publications described the development of inhouse tests for the detection of viral NSP-specific antibodies, the basis of most DIVA tests. Regardless of the test used, relying on the presence of NSP antibodies to detect infection in vaccinated populations (with purified inactivated vaccines) is imperfect, as vaccinated cattle may occasionally seroconvert, particularly after repeated vaccination.

**Projects**

*What activities are planned or underway?*

# Lead Summary 8

| **Title:** | Characterisation of FMD strains |
|---|---|

**Research Question**

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

To understand the bases for functional genomic and predictive genomics.
To develop portable, rapid, low-cost methods and tools for early field characterisation of FMDV.

**Challenge(s)**

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

Development of methods allowing rapid FMDV strain typing.
Identify and deal with recombination in full genome sequencing, including phylogenetic models.
Achieve holistic and unified understanding of functional genomics and deep sequence analysis of diversity.
Validate rapid sequencing tools to be used in the field.

**Solution Routes**

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

There is a range of FMD viruses' strains which differ in terms of virulence. Establishing the genomic differences of the various strains will assist in the identification of virulence mechanisms. Continuous monitoring of FMD sequences will be necessary to ensure the diagnostic are adequate.

**Dependencies**

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

FMDV genome sequencing.
FMDV detection tools.

**State of the Art**

*Existing knowledge including successes and failures*

Next-generation sequencing technologies that allow sequencing of not only the predominant sequence but also minority variants present within a single sample have emerged. This technology has been used to explore within host virus evolution and selection in a way not previously possible, substantially advancing knowledge of FMDV dynamics within the host. A protocol, suitable for usage in a high-throughput laboratory, has been published for whole FMDV genome consensus sequencing.

**Projects**

*What activities are planned or underway?*

# Lead Summary 9

| **Title:** | Identification of new sensitive media or cell line allowing easy and low-cost viral isolation |
|---|---|

**Research Question**

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

To increase the capability to perform virus isolation, which is essential for antigenic characterisation of field isolates and is a critical step in the preparation of conventional vaccine seed stocks. To identify cell cultures for virus amplification.

**Challenge(s)**

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

Cost.
Availability.
Sensitivity.

**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?*

Sample collection/preparation.

**State of the Art**

*Existing knowledge including successes and failures*

Isolation of live virus from the oropharyngeal region is time-consuming and unreliable. Primary bovine thyroid cell cultures have been shown to be the most sensitive for field strains of FMD although sourcing these cells can be problematic particularly in the face of an outbreak.
The ZZ cells (tongue goats cells) are now used instead of primary bovine cells and are as sensitive

**Projects**

*What activities are planned or underway?*

# Lead Summary 9A

| **Title:** | Definition of rapid, safe and low-cost methods for the direct identification of FMDV |
|---|---|

**Research Question**

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

To define portable, reliable, rapid, safe and low-cost methods for the direct identification of FMDV.

**Challenge(s)**

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

Develop technology for FMD rapid vaccine matching.
Define multiplex PCR for vesicular diseases with adequate sensitivity for FMDV detection.
Detect all FMD strains.

**Solution Routes**

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

Develop serotype specific rRT-PCR assay(s) for all virus pools.
Develop TIGR technology for FMD serotyping/subtyping for rapid vaccine matching and monitoring variation of the virus during an outbreak of FMD.

**Dependencies**

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

Primer selection.
FMDV genome.

**State of the Art**

*Existing knowledge including successes and failures*

Virus isolation is considered to be the “gold standard” method for the detection of FMD.
Use of inactivated penside tests to transport the virus (low cost) and to rescue the virus by transfection
A number of RNA detection assays targeting FMDV genome have been developed using reverse transcription loop-mediated isothermal amplification (RT-LAMP), with some detecting single serotypes and others multiple. RT-LAMP was faster, simpler, more cost-effective and at least as sensitive and specific as RT-PCR. Furthermore, it has been successfully used in a portable platform to allow rapid diagnosis in the field. In addition, encouraging results have been obtained from preliminary studies of an RT-LAMP assay coupled with a lateral flow device for use as a rapid, low-cost means for early field detection of FMDV, including from air samples.
Developments in pen-side diagnostics include the evaluation of a portable real-time PCR amplification platform.

**Projects**

*What activities are planned or underway?*

# Lead Summary 9B

| **Title:** | Sequencing of FMD strains |
|---|---|

**Research Question**

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

To implement NGS full genome to understand better the ecology and microenvironment of the virus.

**Challenge(s)**

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

Implement NGS full genome to understand better the ecology and microenvironment of the virus.

**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*

Next-generation sequencing technologies that allow sequencing of not only the predominant sequence but also minority variants present within a single sample have emerged. This technology has been used to explore within host virus evolution and selection in a way not previously possible, substantially advancing knowledge of FMDV dynamics within the host. A protocol, suitable for usage in a high-throughput laboratory, has been published for whole FMDV genome consensus sequencing.

**Projects**

*What activities are planned or underway?*

# Lead Summary 10

| **Title:** | Development of novel non-invasive sample collection methods and low-cost, easy to use, preserving media to collect and transport FMDV safely |
|---|---|

**Research Question**

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

To identify new samples allowing early identification and safe and effective preserving media for FMDV transport allowing high to retrieve high quality virus reducing biosecurity risks.

**Challenge(s)**

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

Isolation of viruses from LFDs.
Develop preserving medium.
Develop low-cost, easy to use medium to collect and preserve FMDV.
Establish a simpler method for RNA extraction.
Validate of the best sample for early detection.
Validate biosafety and biosecurity of transportation of inactivated virus in penside tests.
Investigate swab material impact on sample quality.
Improve sampling technique (probang) and method of preservation.
Validate non-invasive sample collection methods (especially for buffaloes).

**Solution Routes**

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

Investigate technologies that allow sampling and preservation such as dry swabs, or filter paper, or nanoparticles or penside tests that could specifically concentrate and remove virus from inactivating agents.

**Dependencies**

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

**State of the Art**

*Existing knowledge including successes and failures*

Novel sampling methods that show promise include air sampling and baited ropes, the latter may aid sampling in wildlife and swine.

**Projects**

*What activities are planned or underway?*