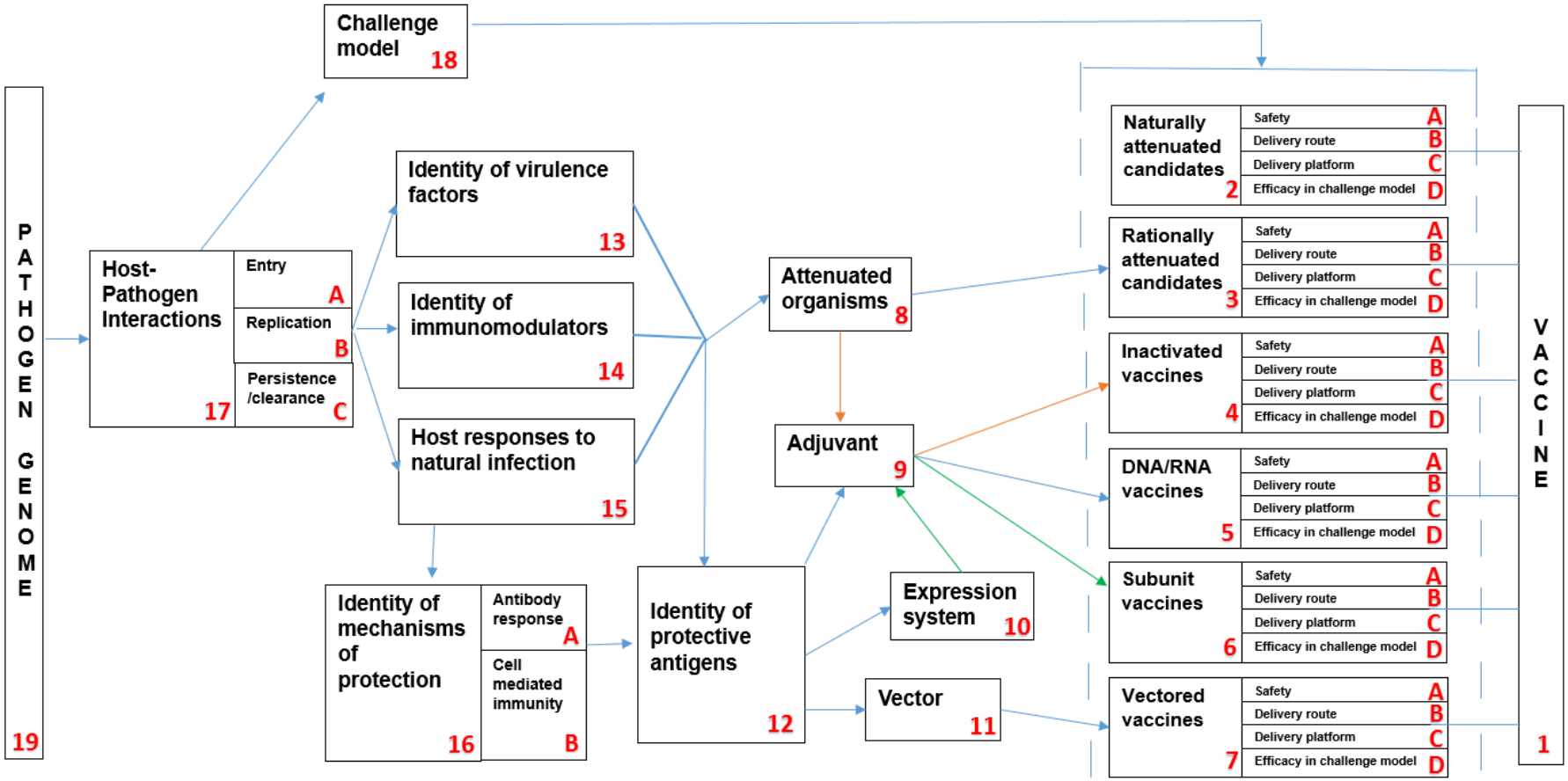
STAR-IDAZ
International Research
Consortium on Animal Health

Roadmap Lead Summaries

Disease/pathogen						
Roadmap type	Vaccine					
Version: Date	V1	31/3/2018				
	V2	8/3/2019				
	V3	18/1/2024				

Roadmap for Vaccine Development



Lead Summary 1

Title:	bTB vaccine

Research Question

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

The development of an effective vaccine against bTB, reducing R_0 to <1 and allowing vaccinated to be differentiated from infected.
Take advantage of the huge effort to develop a vaccine against bTB for cattle to also evaluated trained immunity (i.e. braoad health beneficial effects)

Challenge(s)

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

Vaccines can sensitise animals to current diagnostic tests resulting in false positives.
The current BCG vaccine needs to be validated for preventing onward transmission.
Understanding mechanism of action: reducing population-level susceptibility, reducing disease progression, reducing onward transmission?
Establish immune (biomarker) correlates of vaccine protection.
Epitope sequences across the *Mycobacterium tuberculosis* complex (MTBC) are evolutionarily hyper-conserved, suggesting their recognition is advantageous for the bacterium.
Standardisation of BCG.

In humans, BCG is well known as one of the best inducers of "trained immunity", bringing benefits for health in addition to protection against M. tuberculosis. It will be important to evaluate these potential beneficial effects in cattle as well when designing new vaccines approaches.
The development of a better vaccine based on the rational attenuation of the organism or on purified immunogens delivered by various mechanisms.
Challenge to account for genetic variation within- and between-breed in bTB phenotypes, such as susceptibility, transmissibility, and response to vaccination etc.
Establish if bovine genetics influences responses; does "response to vaccination" show heritable variation?
Undertaking sufficiently powered, controlled, and replicated challenge studies.
Establish optimal delivery route and dose.
Develop effective vaccine(s) for cattle and wildlife.
Establish duration and range of protection.
Consider need for pre-exposure and post-exposure (therapeutic) vaccines for some settings i.e., for wildlife.

Solution Routes

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

Establish protection levels for onward transmission with various candidate vaccine options, **including priming with one vaccine and boosting with a different vaccine**.
Establishing the level of protection given by BCG vaccination as a base line with which to compare other vaccines.
Multi-year, multi-country longitudinal study looking at course of natural infection to analyse with omics technologies to inform the rational design of vaccines.
Mathematical modelling with parameter estimates from experimental and field studies; cattle-only model, multi-host model etc.

Dependencies

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

Validation of BCG in relevant high and low burden settings.
Secure market authorisation and establish efficacy and safety, including food safety.

Development of a novel attenuated vaccine that isn't excreted.
Development of a subunit vaccine.
Development of a DNA vaccine.
Development of a cross protective vectored vaccine.

State of the Art

Existing knowledge including successes and failures

BCG demonstrated to reduce susceptibility and reduce pathology scores in cattle challenge experiments. BCG field trials underway.
BCG in humans provides some protection, particularly in neonates; the neonate effect is seen in other animal models.

Projects

What activities are planned or underway?

Lead Summary 2D

Title:	Validation of BCG efficacy

Research Question

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

BCG needs to be validated in relation to its efficacy in controlling bovine TB for a) its immediate deployment and b) to serve as a baseline against which other vaccines are compared.

Challenge(s)

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

Establish efficacy of BCG in protection against clinical disease and preventing onward transmission.
Understanding mechanism of action: reducing population-level susceptibility, reducing disease progression, reducing onward transmission?
Establish immune (biomarker) correlates of vaccine protection.

Solution Routes

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

Establish the most effective vaccination regimen – dose, number of vaccinations, route of administration and optimal age for administration.
Vaccination, monitoring of immune responses and experimental challenge.
Field trials for onward transmission.
Mathematical modelling with parameter estimates from experimental and field studies; cattle-only model, multi-host model etc.

Dependencies

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

Agreed challenge models.
Correlates of protection.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 3

Title:	Development of a novel attenuated vaccine allowing DIVA

Research Question

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

The development of a novel attenuated vaccine that gives better protection than BCG and allows differentiation of infected and vaccinated animals.

Challenge(s)

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

Which strain of the organism to start with? Reference *M. bovis*, wild-type *M. bovis* or BCG?
Slow growing organisms in culture.
Quality control of biological reagents.
How to rationally attenuate wild-type *M. bovis*? What genes to knock out or in?
How to improve immunogenicity of BCG? Knock out/in which gene(s)?
Identity of genes to knock out as a DIVA marker.

Solution Routes

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

Establishing the immune responses to genetically modified *M. bovis* and BCG
Challenge experiments involving animals vaccinated with candidate organisms.
If demonstrated proof of principle, sufficiently powered, controlled, and replicated experimental or field studies.
Evaluate DIVA test performance characteristics.

Dependencies

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

The generation of GM organisms where the genes for selected virulence factors have been removed and shown to be attenuated in the relevant host.
The genome sequence of *M. bovis* strains.
Efficacy and safety data.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 5

Title:	Development of a DNA vaccine for bovine TB

Research Question

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

The development of an effective DNA vaccine for use on its own or in combination with other bovine TB vaccines in a prime-boost combination.
An RNA-based vaccine against bTB?

Challenge(s)

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

Identity of suitable genes for use as a DNA vaccine.
What are the correlates of protection?
Emphasis on cell-mediated immunology.
Which *M. bovis* gene(s)? Unlike most pathogens, many immunodominant epitopes are likely under purifying selection and hyper-conserved. Are they good targets?
Immunoreactivity is not necessarily protective.
Effective administration in ruminants.
Adjuvant required? If so, which one?

Solution Routes

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

Establishing the immune responses following immunisation with DNA coding for various protective antigens.
Consider in vitro challenge model? Potentially macrophage or granuloma organoid model?
Consider in vivo model, amoebae model, ferret model etc., before calf experiments.
Challenge experiments involving animals vaccinated with DNA fragments identified from experiments looking at the immune responses.

Dependencies

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

Identifying a combination of protective antigens, the genes for which could form part of a DNA vaccine.
Identifying suitable molecular adjuvants to stimulate strong cell-mediated immune responses.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 6

Title:	Development of a bovine TB subunit vaccine

Research Question

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

The development of an effective subunit vaccine for use on its own, or in combination with other bovine TB vaccines in a prime-boost combination, and which didn't interfere with current/future diagnostics.

Challenge(s)

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

Establish immune (biomarker) correlates of vaccine protection.
Establishing if cattle can be vaccinated by bacterial subunits including glycoproteins and carbohydrate components.
Establish whether potential subunit vaccine components can be extracted or synthesised with quality assurance.
Establish efficacy and reproducibility of subunit approach.
Challenge to agree appropriate challenge model.
Challenge to test subunits with sufficient power and replication.
Establishing the immune responses to the various subunits.
Challenge experiments involving animals vaccinated with subunit candidate vaccines that resulted in interesting immune responses.
Establishing that there isn't interference between the various antigens and extant diagnostics.

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?

Better understanding of immune correlates of protection.
Identifying a combination of protective antigens for expression by a suitable expression vector.
The availability of suitable adjuvants to stimulate strong cell-mediated immune responses.
Identifying expression systems that give correct Antigen conformation.
Existence of sufficiently powered, controlled, and replicated proof of principle in vitro and in vivo studies.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 7

Title:	Development of a vectored bovine TB vaccine

Research Question

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

The development of an effective novel vaccine. A replicating organism expressing the correct combination of antigens is more likely to generate the required type of immune response but as it would have a restricted combination of TB antigens it is unlikely to give false positive test results in the standard skin test

Challenge(s)

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

Establish immune (biomarker) correlates of vaccine protection.
Establishing which *M. bovis* antigens to vector.
Establishing which virus vector system most likely to be appropriate.
Establish lack of cross reactivity with extant diagnostics.
Challenge to separate immunoreactivity from protective responses, especially cell-mediated responses.
Generating a strong protective immune response to antigens delivered by a vector.
Provide evidence of vaccine efficacy for replicating and non-replicating vectored vaccine candidates.
Preventing the development of immune responses to the vector.

If principle proven by sufficiently powered, controlled, and replicated vaccine efficacy and safety studies, challenge to obtain market authorisation for a GMO vaccine.
Establish and monitor host range of vectored vaccine; surveillance of non-target hosts.
Establish proof of principle, establish dose, route, and duration of immunoreactivity etc.

Solution Routes

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

Establishing the immune responses to the *M. bovis* antigens following vaccination with the vectored vaccine.
Establishing the immune responses to the vector.
Consider cell biology and transcriptomics experiments in vitro with vaccine candidates and controls.
Consider in vitro and in vivo model experiments to investigate efficacy.
Consider challenge experiments involving animals vaccinated with candidate organisms.
Consider host range of vectored vaccine; consider bioinformatics to investigate host receptor repertoire.

Dependencies

What else needs to be done before we can solve this need?
Identifying a combination of antigens for expression by a vector or a common single antigen to which immune responses may normally be suppressed. Identifying and evaluating a suitable vector for a cattle vaccine. Identifying and evaluating a suitable vector for a wildlife vaccine.

State of the Art

Existing knowledge including successes and failures

Projects *What activities are planned or underway?*

Lead Summary 8

Title:	Genetically modified *M. bovis* (*M. tuberculosis* or other mycobacteria)

Research Question

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

Rational attenuation of *M. bovis* (or other suitable mycobacteria) by removing virulence genes so that it protects against infection but doesn't cause disease.

Challenge(s)

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

That the GM bacterium is stable and can be grown in culture.
Challenge to identify *M. bovis* genes/genome regions to rationally delete.
Challenge to develop rationally attenuated vaccine that doesn't interfere with extant diagnostics, potential for DIVA vaccine development.
Challenge to develop rationally attenuated bTB vaccine that offered protection to all *M. bovis* clones globally.

Solution Routes

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

Removal of one or more virulence genes and establishing viability and virulence and immunogenicity of the resulting organism

Dependencies

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

Identity of virulence factors
Identity of immunomodulatory factors in the bacterium
Identity of immunogens responsible for PPD skin sensitisation

State of the Art

Existing knowledge including successes and failures

Core *M. bovis* genome for viability has been established, ancillary genes identified. Regions of difference (RDs, deletions) mapped within the MTB Complex.

Projects

What activities are planned or underway?

Lead Summary 9

Title:	Identifying suitable adjuvants and/or delivery systems for subunit and DNA vaccine candidates

Research Question

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

Generation of an optimal immune response to the various sub-unit candidates and DNA candidates

Challenge(s)

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

Challenge to understand immune correlates of protection versus immunoreactivity.
Establishing the most appropriate adjuvant or delivery system for various candidate vaccines.
Determine optimal route, dose, frequency of vaccination, duration of protective immunoreactivity.
Challenge to translate and exploit COVID mRNA technology for bTB vaccination.

Solution Routes

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

Immune response to antigens delivered on Nanoparticles.
Immune response following inclusion of various adjuvants with the candidate vaccines.
Molecular adjuvants for DNA/mRNA candidates.

Dependencies

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

Identity of protective mechanisms and immune correlates of protection in vitro and in vivo.

State of the Art

Existing knowledge including successes and failures

Candidate *M. tuberculosis* mRNA vaccines under evaluation.
Several subunit *M. tuberculosis* candidate vaccines and adjuvants in advanced human trials.

Projects

What activities are planned or underway?

Lead Summary 11

Title:	Identifying suitable vector for the expression/delivery of protective Antigens

Research Question

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

Generation of protective responses in the absence of a possible disease-causing replicating organism.

Challenge(s)

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

Challenge to understand immune correlates of protection versus immunoreactivity.
That the *M. bovis* antigens are presented in a similar way to how they are presented by *M. bovis.*
Replicating organisms are likely to give the best immune response but attenuated *M. bovis* may interfere with diagnostic tests or cause disease in immunocompromised animals.
Challenge to demonstrate significant/sufficient protection in vitro and in vivo.
Challenge to obtain market authorisation and approval.
Challenge to provide efficacy and safety data.

Solution Routes

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

Approaches like those for human-adapted *M. tuberculosis* vectored vaccine development.
Generation of genetically modified organisms (viruses or bacteria) expressing possible protective antigens of *M. bovis*.
Preparation of bacterial spores with the possible protective antigens of *M. bovis* adhered to the surface.

Dependencies

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

Identity of the protective antigens.
Local GMO risk assessment.
Optimal dose, route, frequency, and duration of protective immunoreactivity.
Evidence that vectored vaccine doesn't transmit beyond target species.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 12

Title:	Establishing the identity of protective antigens of *M. bovis*

Research Question

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

The identity the *M. bovis* components that the host needs to respond to, to **prevent** and **contain** infection.

Challenge(s)

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

Challenge to understand immune correlates of protection versus immunoreactivity.
Challenge to understand vaccine mechanism; is the candidate capable of blocking infection i.e. sterile immunity, reducing susceptibility, and/or reducing pathogenesis?
Does the candidate offer stage-specific protection?
To identify the antigens that are responsible for protective cellular responses.
Differentiating protective antigens from other antigens that the host is responding to which may be assisting the bacterium evade the hosts' responses at a particular stage of infection.

Solution Routes

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

The identity of the antigens that the host is generating immunoreactivity to and their role in protection (preventing and clearing infection).
Identifying possible protective antigens in the *M. bovis* genome, their expression and trial in challenge experiments.

Dependencies

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

Identity of protective mechanisms operating in immune hosts – the role of cell-mediated immune responses and humoral immunity.
Availability of genome sequences of various M/ *bovis* isolates.
Availability of genome sequences of potential virus vector vaccines.

State of the Art

Existing knowledge including successes and failures

Immune correlates of protection not fully understood for the MTBCand hosts.

Projects

What activities are planned or underway?

Lead Summary 13

Title:	Identification of the *M. bovis* virulence factors that contribute to disease pathology

Research Question

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

Identifying and removal of the factors contributing to pathological changes are essential for generating rationally attenuated vaccines

Challenge(s)

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

Identification of the *M. bovis* virulence factors that contribute to disease pathology.

Solution Routes

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

Generation of a library of transposon-, site-directed or CRISPR-mediated knock-out *M. bovis* where putative virulence factors have been removed and their use in in vitro and in vivo studies, potentially experimental infections.

Dependencies

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

Improved understanding of immune correlates of protection in *M. bovis* in vitro, in vivo, and experimental models.
Improved understanding of *M. bovis* - macrophage interaction – *M bovis* and macrophage gene expression in different in vivo environments (macrophages from naïve and immune hosts)

State of the Art

Existing knowledge including successes and failures

RD mapping in the MTBC has established the BCG attenuation phenotype.

Projects

What activities are planned or underway?

Lead Summary 14

Title:	To establish the identity of the immunomodulatory factors and stealth mechanisms operating in *M. bovis* infections

Research Question

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

Pathogens manipulate the immune responses of the host to survive. Establishing how *M. bovis* manipulates the host's immune response will allow these factors to be removed and thus allow the hosts immune system to react to the organism in a different way, possibly enhancing protection.

Challenge(s)

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

Challenge to understand how TB benefits from major antigens being highly conserved and under purifying selection.
Challenge to separate immunoreactivity from protective immune responses.
The life cycle of human TB exposure and infection comprises several stages; TB cases can progress and regress across this spectrum. Challenge to understand stage-specific immune responses and protection.
Identifying deleterious immunomodulatory factors from ones that would **appear** to be beneficial.
Mycobacteria appear to drive responses in a Th1 direction, so it is essential to establish how this benefits the organism.

Solution Routes

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

Generation of a range of knock-out *M. bovis* where the genes for various immunomodulatory factors or other stealth mechanisms have been removed and their use in experimental infections.

Dependencies

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

Improved understanding of *M. bovis*-macrophage interaction – *M. bovis* and macrophage gene expression in different in vivo environments (macrophages from naïve and immune hosts)

State of the Art

Existing knowledge including successes and failures

Vaccine candidates may act to reduce susceptibility with or without an impact on further, onward transmission; needs to be investigated empirically.

Projects

What activities are planned or underway?

Lead Summary 15

Title:	Identification of the *M. bovis* immunogens responsible for skin sensitisation to PPD

Research Question

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

Infection with *M. bovis* or vaccination with BCG results in positive skin responses to PPD. Identification of factors responsible for the skin sensitisation would allow them to be removed and if not contributing to immunity can be removed from rationally attenuated vaccine candidates.

Challenge(s)

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

Identifying which of a range of factors or their interaction may be responsible for skin sensitisation.
Challenge to understand stage-specific nature of such responses.

Solution Routes

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

Characterisation of PPD – proteomics etc.

Identify the factors responsible for causing skin sensitisation and their genes (fractionation and use in sensitised animals - identify the amino acid sequences – identify the genes).

Dependencies

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

A better understanding of host responses and host-pathogen interactions.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 16

Title:	To identify protective mechanisms in *M. bovis* infected animals

Research Question

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

Identify the mechanisms operating in immune animals, establishing the role of humoral and cell-mediated immunity in **preventing** and/or **containing** infection.

Challenge(s)

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

Identify what is the basis of 'protection'.
Challenge to investigate the stage-specific lifecycle of *M. bovis* infection in its various hosts; for example, is latency an epidemiologically important and consistent feature?
Identify immune correlates of protection, challenge to separate immunoreactivity from protection.
Current tests cannot distinguish exposure from infection.
To identify the role of cell-mediated and humoral immunity in providing protection against infection – passive transfer experiments; identity of cell types responding in recall responses.
To establish the role of the various cell types and cytokine responses in preventing/clearing infection.
To characterise the cytokine and cellular responses in granulomas at different stages of infection – how are infections walled off and become dormant.
Identify disease stage biomarkers.
Identify biomarkers of immunity.

Solution Routes

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

Comparison of the innate and adaptive immune responses of animals that are genetically relatively "resistant" versus susceptible; genetic resistance predictions can now be made.
Consider in vitro and in vivo options for evaluating host reponses.

Dependencies

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

An improved understanding of host pathogen interaction at the level of the infected cell.
Existence of genome sequences of various *M. bovis* isolates, BCG and *M. tuberculosis* etc.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 17

Title:	Host Pathogen interaction in *M. bovis* infection

Research Question

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

An improved understanding of how the host clears or controls infection.

Challenge(s)

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

Current tests generate immunoreactivity but do not distinguish exposure from infection; consequently, a proportion of reacting cattle may have cleared infection. This requires empirical study.
M. bovis infects macrophages which are an important contributor to the immune response so establishing how the bacterium interacts with macrophages is central to identifying the protective mechanisms and how the bacteria evades them.
To enhance the killing of infected immune cells.
Compelling evidence that animals vary in their resistance to *M. bovis* infection; there is heritable and exploitable variation in how animals respond to *M. bovis* exposure.
M. bovis populations can be genotyped into various lineages and clones globally. These genotypes may have epidemiologically relevant phenotypes at the clone level. This remains to be demonstrated empirically.

We need to better understand mechanisms of trained immunity in cattle

Solution Routes

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

Relatively high risk and low risk cattle sires can be identified via quantitative genetics and the TB risk that follows in their daughters. Consider in vitro or in vivo experiments to investigate how these extreme phenotypes handle the pathogen.
TB phenotypes, such as susceptibility and/or transmissibility may be expressed via structural genetic variation or via epigenetics.
M. bovis and macrophage gene expression (transcriptome/RNA sequence data) in different in vivo environments (macrophages from naïve and immune hosts)
Compare response where macrophages are infected with different *M. bovis* strains, BCG and *M. tuberculosis* looking at gene responses of the macrophage and the bacterium.
Comparison of the macrophage-bacteria response following clearance (is infection ever cleared or just walled off?), in latency (this may involve comparative studies involving different breeds or species) and in active infections.
In humans, the live vaccine BCG induces vast health beneficial effects against many pathologies. The mechanism behind that is "trained immunity" where innate cells (macrophages, neutrophils

mainly) establish antigen non -specific "memory" that allows them to clear other pathogens. Similar effects probably operate in cattle and should be measured together with specific protection against Mb. This trait could also help deciding which vaccine should be preferred in case of equivalent protection against Mb.
Analyse mechanisms of trained immunity oi macrophages and neutrophils
Add measures of non-specific effects when analysing the results of protection against Mb

Dependencies

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

Genetic predictions of the relative bTB susceptibility of sires and their progeny.
The genome sequence of various *M. bovis* isolates, including BCG

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?