Mycoplasma bovis science plan

Prepared by the *Mycoplasma bovis* Strategic Science Advisory Group

30 October 2018

Introduction, context and purpose

In July 2017, *Mycoplasma bovis* (*M. bovis*) was discovered in cattle on a South Island dairy farm, prompting the initiation of a national response to the disease. Subsequently, the disease was traced across New Zealand; however, new cases continue to be linked to the original single incursion.

Since the incursion, urgent science needs have been addressed by the operations response team, mainly delivered by Animal Health Laboratories (AHL) at Wallaceville, Wellington; AHL is New Zealand's national veterinary laboratory.

On 28 May 2018, the New Zealand Government, along with the dairy and beef industries, made the decision to eradicate *M. bovis*. The Government announced a $30 million investment over two years for science to support the eradication efforts.

The *M. bovis* Strategic Science Advisory Group (SSAG)[1] was established in July 2018 to support prioritisation of science to speed eradication. The SSAG will provide high-level recommendations to the *M. bovis* Governance Board on the requirements for strategic science and will play a key part in ensuring a high impact and well-aligned science programme.

A collaborative multidisciplinary approach is critical – social, biophysical, and technological expertise will need to be harnessed to address the complexities of the eradication.

Why this science plan was developed

The objective of the *M. bovis* science plan is to build on existing knowledge of the bacteria and disease, along with knowledge from dairy and beef sectors and response teams, to identify the highest priority science needed to eradicate *M. bovis* from New Zealand.

How this science plan was developed

The plan was developed through an interactive, iterative and integrated process of:

- Convening the SSAG for several meetings to identify priority science areas;
- Undertaking a two-day *M. bovis* science workshop in September 2018 that involved approximately 75 experts from science, veterinary, and educational organisations, industry, and government agencies to identify strategic science needs within priority areas;
- Convening the *M. bovis* SSAG to collectively focus the outputs from the workshop into a set of priority research needs, and to draft the plan;
- Circulating the draft plan amongst workshop attendees for feedback; and
- Finalising the science plan and submitting it to the *M. bovis* Governance Board.

Why we are eradicating: The impacts of *Mycoplasma bovis*

M. bovis is an economically significant pathogen of cattle throughout the world. It causes pneumonia, arthritis and mastitis in adult cattle, and middle ear infections, conjunctivitis, pneumonia and arthritis in calves. In addition to these immediately apparent clinical impacts, infection increases susceptibility to other diseases and imposes a subclinical cost on production.

[1] See following website for SSAG membership: SSAG Membership

Disease is typically chronic and responds poorly to treatment with antibiotics. It spreads readily among intensively managed cattle by several pathways. There are no effective vaccines currently available to prevent infection or control its effects. *M. bovis* does not infect humans and presents no food safety risk.

The rapid spread, and the long term and broad range of effects, of *M. bovis*, along with the lack of tools for controlling the impacts of the disease it causes, makes management of this disease particularly difficult. As a result, the impacts of *M. bovis* on farmer and rural community welfare can be severe. A further complication is that currently available diagnostic tests, while effective in identifying an infected herd, are not sufficiently sensitive to identify all infected animals, so it is not possible to ensure that an individual animal from an infected herd is not a potential source of infection when it is introduced to a new herd. It is clear from studies in a number of countries that the best approach to limiting the impact of *M. bovis* in cattle is to prevent its introduction into a herd, as it is not possible to eliminate from a herd without resorting to depopulation.

Therefore, given the limited number of herds infected during this incursion, the best option for the New Zealand cattle industries is eradication.

Summary of research to date

Social science, communication, social and economic impacts

The Response team uses feedback (via the liaison and welfare teams, roadshows and public meetings, and Incident Control Point managers) and media monitoring to understand communications and impacts. This information is used to adapt and evolve Response activities.

The diffusion and communication of *M. bovis* information through farmer social networks is being investigated and incorporated into disease spread models by the Massey EpiCentre.

Work is also being undertaken to analyse publicly available social media data (Facebook, Twitter) of *M. bovis* communications over the response timeline, which aims to understand how information about the outbreak travelled through the industry, and to understand public sentiments towards different aspects of the outbreak response.

A master's degree study (Massey School of Veterinary Science's Farm Services) by a registered veterinarian is using farm and veterinary records and farmer interviews to assess the impact of *M. bovis*-related disease on production. This work will help further inform high-level economic assessments.

National surveillance

A national surveillance programme using bulk milk testing (BMT) for the country's whole dairy supply was commenced in autumn 2018. For the 2018/19 season, spring milk samples have been taken starting four weeks after the start of lactation, and then collected every two weeks for a 12 week period (six PCR samples; one every fortnight, and three ELISA samples; one every month). This provided a detailed survey of dairy properties nationwide. Potential infected properties (IPs) identified from the BMT programme go through an escalation in the detail of testing (with real-time quantitative (qPCR) and ELISA) prior to any additional confirmations being made. The total surveillance programme is expected to be completed within 20 weeks (from August 2018).

In October 2018 farming partners and the MPI response team initiated the next step of phased eradication by starting a survey of 205 calf rearing properties. Testing involves nasal swabbing of calves at a single point in time on randomly selected calf rearing properties across New Zealand. All included properties have no identified connection with infected properties.

To date all infections on beef properties have been connected by animal movements. As with all exotic diseases, if inspectors at meat processors suspect animals are infected they report to MPI (but

suspect animals are not easy to detect via this channel). There has been no change in normal meat inspection processes, as the current process meets all market requirements.

Pathway analysis and genotyping

Investigation of *M. bovis* entry pathways into New Zealand has included risk assessment of potential source areas, analysis of imported products, testing of semen, and genetic analysis of *M. bovis* against publically available database of genomes.

This work has created a phylogenetic tree of *Mycoplasma* strains, and the PubMLST system, which examines 7 house-keeping genes, has been used to identify where the New Zealand isolates are most closely related to international strains of *M. bovis.* From the public databases, there are 106 strain types represented from 13 countries (179 countries were not included in this information due to the lack of publically available data). The New Zealand strain's closest matches (while not identical) are from Europe, USA and Israel.

The completed research has supported the generation of a genomic clock, giving an estimate of time of when the strain arrived in New Zealand (specifically, through the use of Bayesian Evolutionary Analysis Sampling Trees (BEAST) analysis).

The outputs from these analyses are being verified by international experts.

MPI has undertaken an assessment of potential pathways for introduction, with the following pathways being identified as presenting a low but not negligible risk: live cattle, imported frozen embryos and semen, imported used farm equipment, imported feed, imported veterinary medicines and biological products, and other imported live animals.

Laboratory diagnostics and field sampling

Considerable resource has focused on ensuring laboratory diagnostics are robust. This has been a key focus of the Animal Health Laboratory (AHL) team who have worked to ensure accurate assessment of infected properties (IPs). The AHL has focused on finding the best commercial kit for blood and milk sample analysis (with the highest specificity and sensitivity) for measuring *M. bovis.* Screening tests used are commercially available ELISA (enzyme-linked immunosorbent assays) tests and real-time quantitative PCR (qPCR). ELISA is a commonly used laboratory based assay designed to detect a protein, by a highly specific antibody-antigen interaction. qPCR is used to detect particular DNA or RNA molecules in a sample. Key outcomes of work to date include improvement of assay sensitivity by laboratory-based diagnostics, and optimisation of field sampling sites by veterinary diagnosticians and epidemiologists. Properties are defined as infected properties after extensive testing of the herd and confirmatory molecular sequencing.

Epidemiology

Data analysis has been incorporated into multiple areas of the response to *M. bovis*. A data analysis platform specifically designed for Foot & Mouth disease (Standardised Analysis of Disease Information (SADI)) has been converted for *M. bovis*. The platform enables various reports to be generated from response information, which assists the operational team. One example of this is the pipeline diagrams for tracing cattle movements across New Zealand. These reports allow the limited epidemiology resource to be focused on interpretation of analysis, rather than performing the analyses.

Numerous projects have also been undertaken using descriptive data analysis (statistical analysis of the available information). The response teams use large datasets, such as disease prevalence, antibody longevity, and bacteria shedding, to help inform the operations teams. As more data become available, the analysis of this information will continue. The focus is on making the analyses repeatable and sustainable so that they can be rerun.

Work is currently underway to model the disease spread both within herds and between farms; this modelling is used to compare control strategies with the aim of being able to be predictive about disease spread. The initial models are being re-parameterised with updated parameters as per recommendation from the TAG (Technical Advisory Group). The updated models will capture more detail about farm categories, enterprise specific prevalence, and performance of surveillance strategies, information flow and uptake of interventions by various farm systems. It is hoped that model outputs will provide information that can be used to refine day to day activities.

Pathology, clinical signs and impacts of the disease, at animal level

Published literature and overseas experience demonstrates that clinical signs of *M. bovis* typically include mastitis, pneumonia, neurological symptoms in calves, and severe lameness (arthritis). However, New Zealand pathologists quickly identified the limitations of using clinical signs as an indication of disease presence, given the severity of recognisable disease on confirmed infected properties has been low. Clinical signs of *M. bovis* can be similar to those caused by other infections, and it is important that pathologists are able to qualify (differentiate) for farmers and industry whether clinical signs are a result of *M. bovis* or other infections or parasites (such as lung worm).

Disease caused by *M. bovis* is being examined and characterised with use of a technique called immunohistochemistry (IHC). IHC has been developed to help determine the impact of the disease, and will allow assessment of whether *M. bovis* is present within sites of disease, thereby supporting more subtle analysis of the role of *M. bovis* in infected animals.

Nasal swabs, joint fluid extraction, and milk samples are often used overseas for disease detection, and have been used in New Zealand. A new technique of tonsillar crypt swabbing has also been developed specifically for detection of asymptomatic animals as part of the New Zealand response. Development of this technique has been vital: it has high sensitivity compared to many other sites, allowing laboratory confirmation of *M. bovis* in trace animals and exposed herds. Because tonsil swabs are a novel technique, work will be done to help understand how long tonsil infection can last and what it might be able to tell us about animal infection status. As part of this, tonsil swab and serology will be used to evaluate tonsil persistence of *M. bovis* in seropositive animals. It will also be used to compare tonsil persistence of *M. bovis* in exposed animals (by different routes) of different, ages, sex and farm systems.

Science areas

A framework has been developed to identify nine key science areas under which research programmes are grouped (Figure 1).

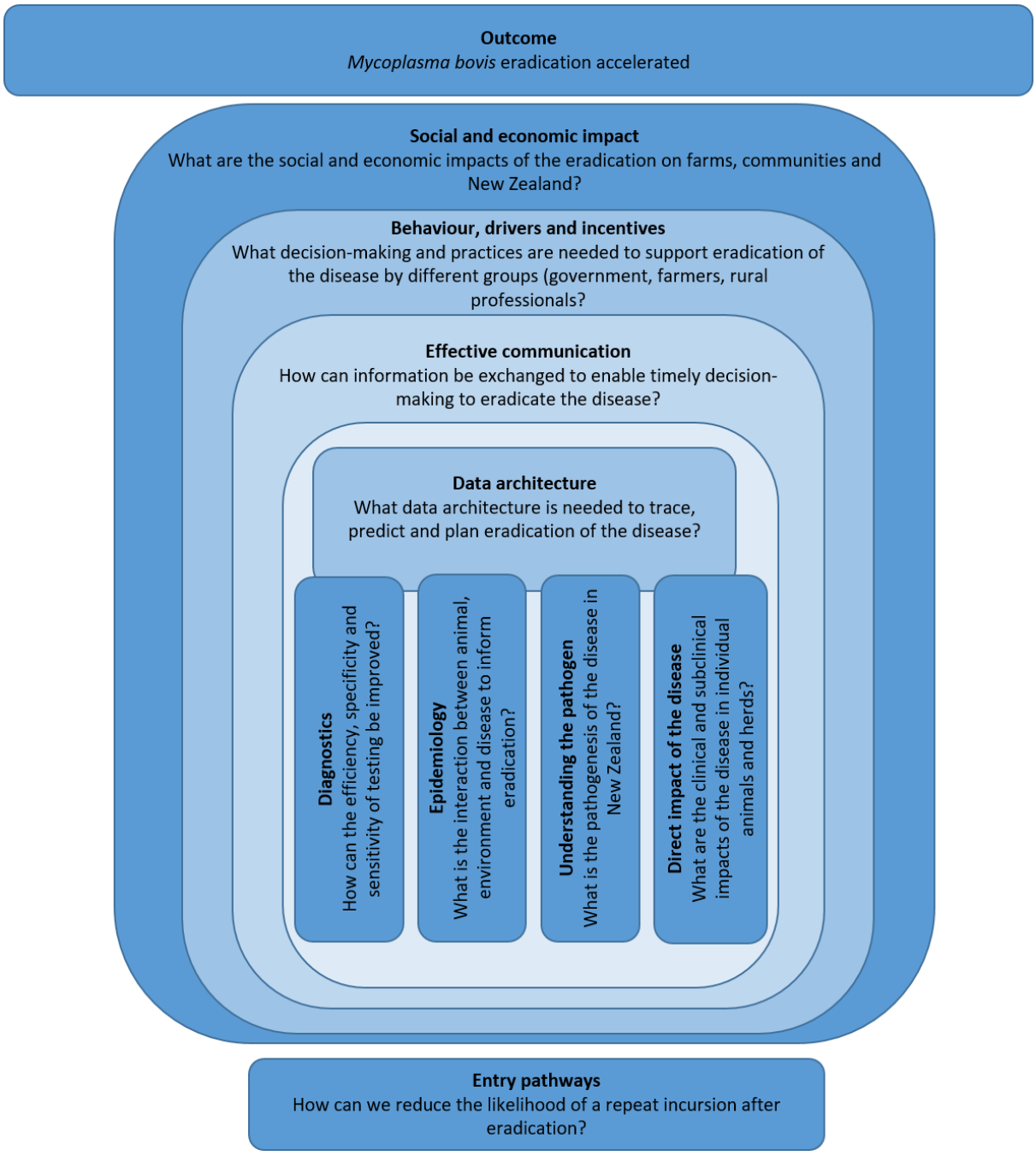

Figure 1: Science outcomes framework

The framework encompasses the four biophysical science areas (vertical boxes) that require research to better enable the eradication of *M. bovis*. To achieve this, data from diagnostics, disease pathogenesis and impacts, and epidemiology will be integrated.

As farmers and rural communities are central to the eradication effort, the research needs in the following sections should be viewed through a farmer and rural community-centric lens.

Understanding how to effectively communicate information on control methods and practices will help to ensure timely and practical decisions and support farmer and rural professional behaviour that enables and accelerates eradication. The latter also recognises that control methods and practices need to fit with farmer and rural professional practices. All science outcome areas are ultimately about effectively mitigating the social and economic impacts of *M. bovis* on farms, communities, and across New Zealand.

Finally, entry pathways for *M. bovis* need to be understood to avoid a repeat incursion.

There are interrelationships across science outcomes and some priority areas could equally be at home in a different outcome area. These cross-outcome relationships need to be taken into account when investment into programmes and eventual contracting take place. Potential science programmes will likely incorporate more than one science outcome area. The ordering of the nine research areas does not represent a particular priority or importance. All areas have been identified as priorities in the eradication.

Priorities

The research needs identified in this science plan were developed through extensive consultation with experts at a science workshop. Prioritisation was based on what workshop attendees believed were most important for eradication.

The prioritisation was further developed by the SSAG and has been categorised in the document as follows:

1 = highest priority

2 = lower priority

Timeframe

The timeframes indicate that we would expect outputs from research by one year, one to two years, or two to five years (from the start of the research programme).

Procurement

Procurement of the research to support accelerated eradication of *M. bovis* will need to proceed with urgency. Procurement will be a mixed model approach and may include direct contracting, closed tenders, targeted requests for proposals, and open tenders. The approach taken will depend on the nature of the research, and the capability and/or facilities required. All proposals will be evaluated by an evaluation panel. Key considerations will include assessment of proposal quality, capability and capacity of teams, and ability to deliver in the required time frame.

Collaborative proposals will be strongly encouraged. Workshops bringing successful researchers together are planned to ensure strong interactions between programmes.

1. Social and economic impact

Aim:

- Ensure key Government agencies, industry organisations, and rural organisations have the appropriate information and tools for analysing and evaluating the social and economic impacts associated with the eradication of *M. bovis*, and can confidently prioritise eradication and support activities to mitigate the negative and enhance the positive eradication-related impacts.

Current state/context:

- The current *M. bovis* eradication program is in full swing. As part of this process, farms are being placed under various levels of constraints, which are resulting in both direct and indirect economic and social impacts for affected farmers, neighbours, support providers and local communities.
- All of these parties are rapidly creating and sharing support information, mechanisms, processes, and networks to deal with the positive and negative impacts.
- Government agencies (e.g. MPI, MBIE, MSD), industry organisations (e.g. Beef+LambNZ, DairyNZ) and rural organisations (e.g. Federated Farmers, Rural Support) are having to make eradication response decisions with limited evidence of the potential positive and negative social and economic impacts on farmers, support service providers, and rural communities.
- Understanding the current and potential social and economic impacts of eradication could aid prioritisation of eradication efforts, including mitigation of negative impacts.

What does success look like?

- The social and economic impacts of eradication are understood, allowing the response to optimise net-benefits.

Priority research needs	Timeframe (years)	Priority	Links to other sections
1.1 Estimating current and potential social and economic impacts of *M. bovis* eradication. **Outcome:** All stakeholders in *M. bovis* eradication understand and can appropriately respond to the potential social and economic impacts on farm teams, communities and response staff.			
a) What are the current and potential economic impacts of *M. bovis* eradication? • On infected and non-infected properties? • On community/regional and at a national level?	1	1	Links to section 9
b) What are the current and potential social impacts of *M. bovis* eradication on farmers, communities and response staff?	1	2	Links to section 9
1.2 Mitigating the negative impacts of *M. bovis* eradication. **Outcome:** All stakeholders are using information on the potential social and economic impacts to optimise the eradication effort and to mitigate negative impacts of *M. bovis* eradication.			
a) What are effective strategies to mitigate the negative impacts of eradication on affected farmers, affected and non-affected communities and response staff?	1	1	
b) How can we optimise eradication response activities to improve the cost effectiveness of the eradication effort?	2	2	Links to section 3

2. Behaviour, drivers and incentives

Aim:

- All members of the farm team and advisors (rural professionals) on the majority of farms with cattle understand their obligations under the Biosecurity Act, and make decisions and implement management practices that support the timely eradication of *M. bovis*.
- All other stakeholders in *M. bovis* eradication (e.g. MPI, industry bodies, livestock transport, and agricultural contractors) implement actions that support farm teams and their advisors in the timely eradication of *M. bovis.*

Current state/context:

- The discovery of *M. bovis* in New Zealand and the decision by Government, DairyNZ, and Beef+LambNZ to eradicate is already changing the biosecurity and animal health advice provided by veterinarians and other animal health professionals on how farm teams should manage their farms, and in particular, advice regarding the isolation and/or movement of stock within and between farms.
- Livestock tracing using information in NAIT has highlighted unsatisfactory historical compliance, which has resulted in unacceptable time lags in tracing. This has led to regulatory changes in NAIT, and increased awareness of its benefits.
- Just as we need to understand the *M. bovis* pathogen and its epidemiology specific to the New Zealand farming context, to support eradication we also need to understand how and why farm teams and their advisors are changing farm management in the presence of *M. bovis*.
- Methods and practices to control the spread and manage the eradication of *M. bovis* are more likely to be implemented by the farm team and advisors when the practices:
    - Are easily adaptable to fit with current farm management;
    - Are sufficiently incentivised; and
    - Enabled by the actions of all other stakeholders in *M. bovis* eradication.

What does success look like?

- Every property with cattle:
    1. Has an effective biosecurity strategy and implementation plan; and
    2. Implements and monitors the performance of these.
- Farm team members and industry understand the value of, and undertake the timely recording of data.
- Good biosecurity practice, with continuous improvement, becomes the norm on the majority of farms.

Priority research needs	Timeframe (years)	Priority	Links to other sections
2.1 Developing an integrated approach to implementing an effective biosecurity plan for the timely eradication of *M. bovis* from New Zealand farms. **Outcome:** All stakeholders in *M. bovis* eradication undertake their obligations under the Biosecurity Act, and implement biosecurity plans that reinforce the collective effort for timely eradication.			
a) Understanding current farm biosecurity practice as a starting point for developing effective biosecurity plans: What is the incidence, nature, effectiveness, and drivers for current biosecurity practice and high-risk behaviours by farm teams and the biosecurity related advice they receive from their advisors (rural professionals)?	1	1	Links to section 3
b) Developing and implementing effective biosecurity practice: How do we develop, test, evaluate and implement widely-agreed biosecurity plans that have rapid, widespread adoption and implementation by all stakeholders in *M. bovis* eradication?	1	1	Links to sections 1, 3, 5, and 7
2.2 Increasing the effectiveness of livestock tracing using NAIT to support timely eradication of *M. bovis* from NZ farms. **Outcome:** All stakeholders are effectively implementing NAIT to support timely eradication of *M. bovis*.			
a) Understanding current adoption rates of NAIT: What is the incidence, nature, effectiveness, and drivers for current use of NAIT?	1	1	Links to section 4
b) Developing and implementing effective plans for widespread adoption of NAIT: How do we develop, test and evaluate approaches to increase the effective adoption of NAIT?	2	1	Links to section 4 and 7

3. Effective communication

Aim:

- All members of the farm team, rural professionals, and associated rural community of all farms with cattle are receiving relevant information, from trusted sources, and in a format that enables them to confidently make timely and effective decisions that support the eradication of *M. bovis*.

Current state/context:

- The rural sector is not sufficiently aware of progress towards eradication and misinformation is spreading through some networks.
- We don't have a good understanding of how to identify and utilise less formal channels of communication.
- We don't understand how current communications efforts influence adoption of practices and decision-making.

What does success look like?

- Appropriate audiences and corresponding communication channels are identified and effectively engaged for knowledge exchange and uptake of good biosecurity practices.
- Sector communication and decision-making complements and supports the eradication of *M. bovis*.

Priority research needs	Timeframe (years)	Priority	Links to other sections
3.1 Audiences and their information needs. **Outcome:** We know who we need to talk to, how to access them, and what information they need.			
a) Identifying audiences, channels to those, and their information needs: Who are the key groups that MPI and other stakeholders need to target, how are these best addressed, and what do they need to know?	1	2	*All*
3.2 Sources and channels. **Outcome:** Clear understanding of where each stakeholder group gets their information from.			
a) For each stakeholder group, what are the most effective sources and channels for knowledge exchange and uptake?	1	2	*All*

4. Data acquisition and architecture

Aim:

- Better understand effectiveness of existing data sources for traceability, predictive value, and scenario planning.
- Biosecurity data infrastructure is fit-for-purpose for handling incursions. This includes provision for greater accessibility for researchers before, during, and after incursions.

Current state/context:

- New Zealand as a whole collects a large volume of farm and animal level information via various central government, regional government, industry specific and farm specific platforms and routes.
- Potentially these data, at least at the farm level, cannot be fully realised as a single farm identifier is not present nor defined in New Zealand.
- Animal level data related to movement history and location is collected (for cattle and deer) in the NAIT application, however compliance with movement reporting and animal registration has emerged as a gap. Even when compliance is good, there are barriers to aligning and integrating existing health, treatment, and production information from other firm or industry systems at the animal level with movement history.
- There are public and private identifiers in use, as an example, milk companies have supply numbers that must be spatially linked to property identifiers.
- The national agricultural census (5 yearly) and survey (annually) uses a sample frame and definitions which are very hard to match against existing national property databases to obtain animal population information.
- The lack of common data definitions, tagging and sharing rules, and open APIs has resulted in limited system-wide integration across different platforms and solution offerings. As a result, farmers are required to enter farm-level data multiple times.
- There have been limited individual direct economic incentives or benefits accruing to farmers for ensuring continual data capture and compliance.
- While this deficit is present in all production systems (placing biosecurity efforts at risk), the execution of a successful proof of concept for cattle would provide good momentum to expand to other sectors.

What does success look like?

- A frictionless, farmer-centric system to record animal and farm records that is used by all cattle owners, based upon an open data architecture and common rules that interacts with existing national systems, and allows for timely and effective analysis as well as making data security and privacy a priority.

Priority research needs	Timeframe (years)	Priority	Links to other sections
4.1 Farmer centric data architecture that enables seamless and efficient integration. **Outcome:** Ensure that the data capture mechanisms, instruments, and processes are farmer and rural professional centric, incentive compatible, enable efficient data acquisition, and provide positive market benefits.			
a) How do we create a frictionless farmer-centric system that creates incentives for farmers' participation and provides animal and farm records/data based on common language, rules, and definitions?	1	1	Links to section 2

5. Entry pathways

Aim:

- Understand entry pathways into New Zealand to reduce the likelihood of a future incursion.

Current state/context:

- The pathways report released by MPI made the following conclusions:
  - There are seven potential introduction pathways (imported live cattle, imported frozen semen, imported embryos, imported feed, imported used farm equipment, and other imported live animals).
  - Some pathways are more likely to have caused the outbreak (i.e., imported frozen germplasm) than others (imported live cattle, imported used equipment).
  - The seemingly one-off nature of the outbreak might be explained by a failure of existing border measures to prevent entry or entry through an unregulated/illegal pathway, the detail of which is currently unknown.

What does success look like?

- New Zealand has robust import health standards that support trade and which include evidence-based risk management measures for bovine semen and embryos.
- We understand the risk of *M. bovis* presence and transmission through germplasm.
- Likelihood of disease re-entry post-eradication is reduced.

Priority research needs	Timeframe (years)	Priority	Links to other sections
5.1 Improve surveillance strategies. **Outcome:** Optimised surveillance to increase the chance of detecting *M. bovis* before entry to New Zealand.			
a) Risk management: How can we improve monitoring around risk pathways? • Design and make recommendations to optimise surveillance, including recommendations for the six identified risk entry pathways (imported livestock, semen, embryos, used farm equipment, feed, veterinary medicines and biological products).	2	1	Links to section 7
5.2 Improve diagnostics for robust border management that facilitates trade. **Outcome:** Improved detection of infected source animals and germplasm imported to New Zealand to inform the rapid risk assessments for *M. bovis* in bovine semen and embryos.			
a) What is the risk of presence and transmission of *M. bovis* in semen and embryos, to inform rapid risk assessment?	1	2	Links to section 6
b) How can we improve diagnostic tests to enhance border management?	1	2	Links to section 6
c) What is a suitable testing protocol for bovine semen and embryos and live cattle?	1	2	Links to section 6
5.3 Investigate strategies for bovine germplasm treatment to reduce risk of importation. **Outcome:** Suitable treatment of infected germplasm.			
a) Are current treatments sufficient to prevent entry of *M. bovis* in germplasm?	1	2	
b) What additional treatment strategies could reduce the risk of entry of infected germplasm?	1	2	
c) Compare and validate pathways to reduce the risk of *M. bovis* re-entry.	1	2	

6. Diagnostics

Aim:

- Improve existing diagnostic assays by increasing the sensitivity and specificity of sampling procedures and tests.
- Develop novel assays to identify disease cases in the absence of seroconversion or *M. bovis* shedding.

Current state/context:

- *M. bovis* infections can be diagnosed by bacteriological culture, but this is difficult and time consuming. False-negative results can be common. Serological methods and molecular identification methods are faster and more samples can be tested.
- Serological tests are only effective once an animal seroconverts, which may occur days weeks post-infection.
- The major challenge to current antigen and molecular detection tests is the intermittent shedding of *M. bovis*, a result of the bacteria's ability to localize in tissues that are not easily accessible to sampling.

What does success look like?

- Ultimately, "better diagnostics" will include a better understanding of:
    1. The influence of sampling time, i.e., "peak shedding" or "max response";
    2. Performance of existing diagnostic tests against different analytes (blood, milk, tissue);
    3. The best method of detection for each sample type.
- Increase the diagnostic sensitivity of bulk milk testing (by ELISA and/or PCR) through:
    1. Optimised sample processing (enrichment); and/or
    2. Detection methods with higher sensitivity.
- Improved sensitivity and specificity of diagnostic tools at the herd level (dairy and beef).
- Availability of diagnostic tools to identify "the last positive animal" (supported by risk prediction tools); availability of diagnostic tools to monitor freedom from *M. bovis*.
- Identification to the species level is important as multiple *Mycoplasma* species can infect cattle that are either part of the resident microbiome or can cause pathogenicity.

Priority research needs	Timeframe (years)	Priority	Links to other sections
6.1 Increase the diagnostic sensitivity of bulk milk testing. **Outcome:** Significantly increasing the sensitivity of bulk milk testing (by ELISA and/or PCR) in practice.			
a) How do we develop methods to enrich *M. bovis* DNA from milk samples (i.e., increase PCR sensitivity)?	1	1	Links to section 7
b) How do we develop an ELISA method with increased sensitivity for the detection of *M. bovis*-specific antibodies?	1	1	Links to section 7
6.2 Develop a strategy for herd-level testing of beef cattle. **Outcome:** A suitable strategy for herd-level beef testing.			
a) How do we develop and validate a strategy for testing beef cattle at herd-level?	2-5	1	
b) How do we develop risk-prediction models that can be used as decision-making tools to identify herds and/or individual animals that are at risk of being infected and as such are "worth" testing?	1-2	2	

Priority research needs	Timeframe (years)	Priority	Links to other sections
6.3 Improve testing strategy. **Outcome:** Minimise the time a farm spends under a Notice of Direction.			
a) How do we develop and validate faster testing procedures, including sampling, testing, interpretation, and reporting to minimise farmer uncertainty?	1-2	1	
6.4 Develop novel: diagnostic tools for individual animal testing and methods of detection for bulk milk. **Outcome:** Diagnostic tools sufficient to test at animal level and novel bulk milk methods of detection.			
a) How do we develop, adapt, and validate novel diagnostic methods (beyond PCR and ELISA) with sufficient specificity for individual animal testing, including in the sub-clinical or latently-affected animal?	2-5	1	Links to section 9
b) How do we develop novel bulk milk methods of detection of *M. bovis* presence in the host, even when not shedding and seronegative (e.g., bacterial or host miRNA)?	2	1	
6.5 Develop a test for detection of live *M. bovis* in environmental samples. **Outcome:** Live *M. bovis* can be detected in the environment, if present.			
a) How do we develop and validate a test for detection of live *M. bovis* (specific to New Zealand strains) in environmental samples, including sampling?	1	1	Links to section 8
6.6 Availability of test strategies and methods to establish freedom from *M. bovis.* **Outcome:** Proof of freedom can be determined.			
a) How do we develop methodologies that establish freedom from *M. bovis* in New Zealand?	2-5	1	Links to section 7

7. Epidemiology

Aim:

- Understand the interaction between animal, environment and disease at a level that can inform risk based surveillance and provide assurance that surveillance following eradication of the last known infected place would identify any positives (should they be present).

Current state/context:

- Response epidemiology information is currently analysed in a custom built platform designed for *M. bovis* epidemiology analysis. There is some data integration that should be undertaken to expand the type of analysis possible.
- Focus on tracing of every suspicious animal movement and the use of molecular genomics has allowed a coherent and somewhat encouraging picture to emerge that supports a single strain incursion in early 2016.
- Molecular genomics, using whole genome sequencing and evolutionary modelling, has also helped determine likely transmission pathways and evolutionary dynamics.
- National bulk milk testing is ongoing.
- The progression of disease within different farm types and management systems and the progression and behaviour of the disease in individual animals is not fully described and adds uncertainty.
- Integration and analysis are both ongoing as is the laboratory testing of the backlog of biological samples.
- Future work in the epidemiology area is contingent on all the lab testing results being available, integrated and analysed.

What does success look like?

- All linkages between infected properties (including knowledge of genomics and how properties became infected) are identified. This will provide additional confidence in the single point source epidemic hypothesis.
- Ongoing national surveillance strategy is fit for purpose and optimised for eradication, with risk factors for infection, disease, and spread identified.
- There is confidence in the testing strategy to determine freedom from *M. bovis*.
- New epidemiological work is seamlessly integrated into the response work.

Priority research needs	Timeframe (years)	Priority	Links to other sections
7.1 Optimising surveillance. **Outcome:** Surveillance strategies build on the work currently being done by the response to identify farms to test.			
a) What is the performance of the current New Zealand surveillance strategy as a whole? How can existing surveillance streams and results be amalgamated and sensitivity of the entire system estimated, including the provision of a platform (preferably using existing response data architecture) in which the up-to-date results can be regenerated on an ongoing basis throughout the eradication programme?	1	1	Links to sections 4 and 5
b) What are the current gaps in the surveillance system and what are options to overcome these in a way that is fully integrated in the day-to-day response functioning?	1	1	
c) How do we optimise current and long-term herd-level testing strategy (ELISA/PCR/other use of existing tools – see bullet point 5 under "current state" above)?	1	1	Links to section 6

Priority research needs	Timeframe (years)	Priority	Links to other sections
d) What is the potential for national risk-based surveillance (vs general scanning surveillance based on random sampling), linking closely to current work on this in the beef sector?	1	1	
e) Should risk-based criteria be applied to movement-linked properties to prioritise these sites for operational visits, including a cost-benefit analysis?	1	1	
7.2 Efficiently finding infection. **Outcome:** Effective understanding of infection spread to help find at-risk properties.			
a) How do we understand our animal movements, and best use our data? Can NAIT data gaps be overcome?	1	2	
b) What are the risk factors for spread at farm and mob levels (using existing data integration projects)?	1	2	
c) What are the characteristics of a high-risk herd (using existing data from Infected Properties)?	1	2	
d) What are the herd-level risk factors for clinical disease versus infection?	1	2	
e) What are the within-herd and between-herd infection dynamics? • within-herd contacts between animals in different farm and production types; • within and between mobs on farms (cattle only); and • the contact patterns represented by conveyors between farms.	1	2	Links to section 2
f) What is the within-herd prevalence curve through time?	1	2	
7.3 Define 'freedom from *M. bovis*'. **Outcome:** Surveillance provides confidence of freedom from *M. bovis*.			
a) How can testing strategy be optimised to determine freedom from disease in a sector-specific manner (accounting for differences in production types), including determination of aspects such as appropriate sample size, type of test, and sample timing and the inclusion of risk-based strategies if appropriate?	2-5	1	Links to section 6
b) How can we aggregate all of the existing surveillance streams to estimate confidence of freedom from disease?	2-5	1	

8. Understanding the pathogen

Aim:

- Control methods and disease risks are identified by determining the mechanisms and infection routes that *M. bovis* uses to survive, and, potentially, evade its eradication.

Current state/context:

- Since *M. bovis* was detected in New Zealand in July 2017, gene sequencing has identified that just one strain is present.
- DNA mutation analysis indicates the strain probably entered the country in early 2016.
- The eradication policy is based on slaughter of infected herds with DNA-positive diagnostic tests.
- It is known that *M. bovis* is spread hematogenously in the host and even infected semen can be a source of infection.
- Although *M. bovis* lacks a cell wall, it is capable of producing biofilms, which aid its survival in the environment.
- To ensure the *M. bovis* eradication programme is successful and not prolonged and that re-introduction of the organism does not occur, it is essential that:
    - the survival of *M. bovis* in the environment and potential infection routes defined;
    - the pathogenesis of the organism and the host response is understood.

What does success look like?

- Understanding of the dissemination and predilection sites of *M. bovis* in the host.
- Identification of reservoirs of infection in the host and in the environment so that effective control measures can be implemented.
- Effective protocols for cleaning and disinfection of *M. bovis* contaminated farms, their land and animal waste.
- At the individual cow level, we have a greater understanding of the pathophysiology and host-agent interaction of *M. bovis* in exposed and infected animals to optimise sampling and testing at the individual animal level (including molecular and immunological sampling methods, timing of sampling, and optimal sampling for all response surveillance streams).

Priority research needs	Timeframe (years)	Priority	Links to other sections
8.1 Identify potential reservoirs of infection. **Outcome:** Comprehensive understanding of infection reservoirs for *M. bovis* in New Zealand.			
a) What is the survival time/persistence of viable *M. bovis* in the New Zealand environment?	1	1	Links to section 6
b) What cleaning and disinfection methods can be designed to eliminate *M. bovis* from contaminated farms, their land and animal waste?	1	1	Links to section 6
c) Over time, what is the relationship between genotype and phenotype that may impact on infection detection, cleaning and disinfection and potential reservoirs?	1	1	Links to sections 6 and 7
8.2 Infection models in hosts. **Outcome:** Comprehensive understanding of infection in hosts.			
a) What is the minimum infectious dose for *M. bovis* to: • persist in the host; and • cause clinical symptoms?	1	1	Links to section 9
b) What are all the potential routes of *M. bovis* infection?	1	1	Links to section 5

Priority research needs	Timeframe (years)	Priority	Links to other sections
8.3 Pathogenesis and host response. **Outcome:** Understand pathogenesis and host response to assist with developing diagnostic tests and efficacy of surveillance.			
a) Either on farm or by using an infection model, what is the: • dissemination of *M. bovis* within the host; • transmission dynamics between animals; • interactions between *M. bovis* and the host that stimulate clinical symptoms and the intermittent shedding of *M. bovis;* • pathophysiology of infection, including persistence of the agent in various tissues; • the host's immune response to infection, including how *M. bovis* induces or evades immunity at the cow level and consideration of the kinetics of the immune response and cofactors involved; and • *M. bovis* pathogenesis, its mechanisms and potential virulence factors?	2	1	Links to sections 6 and 9

9. Direct impact of the disease

Aim: *Conduct a natural infection study, the time period of which will be limited by MPI and farmer agreed animal cull dates, in order to:*

- Understand the clinical and sub-clinical impacts from disease caused by *M. bovis* in individual animals and in affected cattle herds; defined at both the part-herd level (affected cohort) and the cattle herd as a whole. Determine the subclinical costs of infection with *M. bovis*, including the effects on milk yield and milk quality, as well as effects on weight gain in beef cattle.

Current state/context:

- At present there are widespread beliefs about the size of the impact *M. bovis* on our cattle herds. These beliefs stem from two main misconceptions:
    1. That the significant impact seen on Infected Property 1 will be the same on other Infected Properties; and
    2. That disease impacts can often be seen in growing animals (calves). The validity of this latter finding is unconfirmed. A small number of intensive investigations on affected properties have reported that illness observed has been caused by endemic agents (rather than *M. bovis*). Thus, the true impact of disease, under different farming systems in New Zealand, has not been defined.
- Understanding impact has relevance to understanding the epidemiology of disease (i.e., what factors govern the occurrence of disease and thus the ability to detect it). Thus, impact relates to the sensitivity of surveillance at the clinical level, herd level (through analysis of data and identification of risk factors that influence its occurrence) and at the laboratory diagnostic level (what factors influence disease, presence and excretion of the agent; and thus consequential detection through laboratory methods).

What does success look like?

- Disease impact from *M. bovis* has been quantified under a number of risk factor settings.
- Key risk factors for disease impact have been identified that aid in our understanding of the epidemiology of disease and improves the sensitivity of surveillance through taking account of these factors in surveillance design.

Priority research needs	Timeframe (years)	Priority	Links to other sections
9.1 Prospective risk factor study. **Outcome:** Impact of the disease at the individual cow, group, and whole herd level is understood to support social licence for eradication.			
a) Prospective risk factor study that measures the clinical and sub-clinical impact of disease from <u>natural</u> infection (including effect on milk yield and milk quality, and on weight gain, and the duration of these effects) at the individual cow level, and the part and whole herd levels over time. Researchers will be required to work with MPI Response to identify suitable existing IPs to study. Study time periods will be determined by the cull date agreed between individual farmers and MPI.	1	1	