Report of the Technical Advisory Group on the *Mycoplasma bovis* programme

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1 Executive Summary

The TAG recognises the progress made by the programme. From a situation in mid-2019 where there were more than 50 known infected herds, the number of known infected herds had dropped to 1 by September 2020. The efforts of all involved in the programme are commended for this achievement. The recent detection of a cluster in Canterbury is not unexpected as is the case at this same stage in any national eradication programme, and the location of this cluster is also not unexpected given the high historical incidence in this area. However, the number of herds involved in this cluster suggests that ongoing animal movements remain a challenge for the programme.

Network surveillance has improved since the last TAG report. Over the duration of the programme, there have been demonstrable reductions in intervals from identification to tracing, to placement of Notices of Direction (NOD) and to resolution. Automation of backward and forward tracing of the NAIT data has resulted in substantial time-savings and improvements in operational performance. Re-assessment of high-risk historic cases using the updated casing and tracing tools (the proposed “Network Assurance Project”) is supported by the TAG to minimise the risk that any previously undetected animal movements may have been missed.

The two background surveillance streams, bulk tank milk (BTM) testing and the National Beef Cattle Surveillance (NBCS) programme, are contributing significantly to the *M. bovis* programme. BTM testing has detected a number of infected dairy herds that had not been identified through network surveillance. The national coverage of almost all dairy herds in New Zealand makes this a very effective tool for detecting infected dairy herds. The specificity of the bulk tank milk ELISA testing, while extremely high, is less than 100%, which results in some uninfected herds being test positive. However, relatively few herds are testing positive, so the investigatory workload is manageable. The majority of BTM test positive herds have subsequently been demonstrated to be free of disease upon further animal level testing. This is an expected outcome and will continue throughout the life of the programme.

The introduction of the three surveillance streams for beef herds is a significant step forward for the program. The likely low within-herd (i.e. animal level) level (prevalence) of infection, poses significant challenges in terms of demonstrating freedom from infection, particularly in smaller herds where the small number of animals tested limits confidence about the true infection status of those herds. If the number of animals selected for sampling within herds is based on an assumption that the within-herd infection prevalence is 5% (i.e., the ‘design prevalence’ = 5%), and in reality the prevalences are actually lower, then our confidence that any given herd (and hence the country) is free of infection is reduced. Notwithstanding this constraint, the low numbers of animals testing positive in beef breeding operations, at sampling of animals at slaughter, and upon entry to a beef feedlot provides some

assurance that infection is not widespread in the beef sector. However, more work is required to better understand the *M. bovis* situation in the beef sector, and amongst groups of non-lactating animals more generally. The specificity of the animal-level test appears higher than had been expected, with the net effect that there are fewer herds with false positive results and less disruption to farming operations than had been anticipated originally.

Evidence from the network and background surveillance (bulk tank milk and NBCSP), as well as from the genomic analyses, that there are not widespread and unrelated infected herds, supports the previous inferences that one or a small number of *M. bovis* introductions occurred relatively recently (~2015).

An *M. bovis*-positive feedlot will need to be depopulated prior to declaration of provisional freedom from infection. This needs to be planned carefully to minimise economic impact and the risk of subsequent reinfection. Prior to depopulation, biosecurity measures on the property need to be carefully managed to minimise the risk of spread to other properties.

The programme has developed a high level strategy around the transition from delimitation to declaration of provisional freedom from infection, and finally declaration of freedom from infection, based on OIE guidelines. Establishing clear milestones to be achieved prior to declaration of provisional freedom of infection is very important, as it will inform stakeholders and the farming community more generally. This planning should include criteria for declaring when each milestone is achieved. The TAG recommends that transition of status criteria be defined based on epidemiological data, rather than on specific calendar dates. Additionally, the appropriate metrics to monitor progress during delimitation and during the provisional freedom from infection phases should be defined and implemented. Detailed planning of the surveillance efforts to be used during the provisional freedom from infection phase needs to be undertaken.

The TAG supports the use of scenario tree modelling to support planning and subsequent analyses of surveillance data. It will be important to revise and update the key assumptions and input parameters as new information is gathered and undertake a sensitivity analysis using lower design prevalences for the beef surveillance streams. The TAG supports additional evaluation of disease surveillance methods using different analytical approaches (e.g. spread simulation modelling). Greater confidence in the proposed surveillance approaches would be achieved if different modelling approaches reach broadly similar conclusions about the optimal relative weightings of the various surveillance streams and degree of confidence in national freedom from infection.

As noted above, during the provisional freedom from infection phase, it is expected that there will be test positive herds (as no testing systems are perfect). Hence

systems and resources will be required on an ongoing basis to rapidly and effectively investigate test positive herds.

2 The TAG recommends that

1. whole genome sequencing of *M. bovis* isolates continues and these data are incorporated into operational databases as rapidly as possible to enhance understanding of transmission routes and aid operational decisions.
2. the processes and stop/go points for the proposed Network Assurance Project be more clearly defined, including estimating the number of herds likely to be retraced, and the potential impacts on resourcing and on the farmers involved.
3. a clear plan be developed for destocking and restocking the infected beef feedlot to ensure that the property is free of infection before declaring provisional freedom from infection.
4. additional risk-based surveillance based on region and/or contact networks be implemented for non-dairy farms i.e. farms that are not under bulk tank milk surveillance, independent of sampling associated with TB testing.
5. a risk assessment of animal movements between the non-dairy and dairy sectors be undertaken. This should use whatever data are available including for example NAIT and EDIR data and include an assessment of the risk from grazing dairy replacement heifers on farms where non-dairy animals are also present, co-grazing dairy cows on support properties during the non-lactation period, and movement of beef sires into dairy herds.
6. an assessment be undertaken of likely within herd (i.e. animal level) prevalences in infected non-dairy herds (i.e. the proportions of animals that are infected within infected herds) to inform the animal level design prevalence required for surveillance during the provisional freedom from infection phase. This should be complemented by a sensitivity analysis of surveillance efficacy at a range of design prevalences.
7. national bulk tank milk surveillance should continue, and any reduction in the proportion of herds or frequency of testing be carefully considered. The most recent estimates of diagnostic sensitivity should be included in the scenario tree modelling and in the economic modelling which may result in the cost benefit of the bulk tank milk surveillance being greater than the current modelling suggests.
8. a desktop risk assessment of the likelihood of *M. bovis* infection in herds not supplying milk to milk processors, and hence not being captured within the current bulk tank milk surveillance system, should be undertaken.
9. automated reporting of disease metrics be implemented as rapidly as possible, and the analytic capabilities of databases be further developed.
10. additional fields be added into the databases including genome sequencing data (e.g., clade and subclade), more detailed information on the enterprise type(s) on a farm, and likely (but not confirmed) sources of infection.

11. the laboratory requirements for the provisional freedom from infection phase of the programme be clearly defined, and appropriate laboratory contracts put in place. Additionally, consideration should be given to routinely testing split samples in addition to the current proficiency testing.
12. detailed planning of the transitions from the current delimiting phase to provisional freedom from infection, and from provisional to declared freedom from infection should be undertaken, including definitions of the epidemiological criteria to be met before these transitions are undertaken and an associated communication strategy.
13. scenario tree modelling be used to support surveillance planning. The potential benefit of surveillance of other potential infection pathways (for example, beef bulls used as terminal dairy sires, dairy heifers being grazed remotely from herds of origin) should be assessed in the scenario tree model. The assumptions within the *M. bovis* scenario tree model should be updated using:
    i lower herd level and within herd design prevalences,
    ii the most recent estimates of diagnostic sensitivity and specificity of tests used in the different surveillance streams (i.e., bulk tank milk, beef breeder, etc.).
    iii cost/benefit analysis of additional surveillance .
14. other modelling tools should be used to underpin assurance of freedom from infection through surveillance.

3 Current situation and context for this report

Mycoplasma bovis was first identified in New Zealand in July 2017, and in mid-2018 the decision was made to eradicate this pathogen. At the current time (5 March 2021), there are 10 known infected herds, from a total of 261 infected herds (cases) detected to date (Update 237). The rate of detection of new cases has generally been declining (suggesting that the incidence of infection is declining) despite increased testing, with the net effect that the number of known infected herds has also been declining (suggesting that the herd level prevalence of infection is also declining).

Assessment of the currently available data, including the casing and tracing networks, genomic analyses of isolates from infected herds, and the background surveillance of both the dairy and beef industries, continues to support the assumption that there was a single introduction (or a small number of very closely related introductions) of *M. bovis* around 2015. With nearly 700 isolates now sequenced from ~40% (104/260) of the infected herds, there is increasing confidence that we are only dealing with very closely related isolates, making it highly unlikely that *M. bovis* was present historically in New Zealand. While not every infected farm has been linked with a known source of infection, the lack of recorded movement data in NAIT and/or an inability to recall all animal movements is

the most plausible explanation for these missing links. Genomics provides valuable information about potential sources of infection when there is incomplete tracing data, confirming tracing data and excluding animal movements that did not result in transmission of infection. The predominant pathways for transmission of *M. bovis* in New Zealand are movement of animals or milk. On this basis, achieving freedom from infection is feasible, as long as infected animals and herds are identified rapidly following introduction of infection, and movement controls are applied prior to onward movement of infected animals. Movements of infected animals from infected herds before those herds are identified poses the major risk to achieving freedom from infection.

Since the last TAG report (18 October 2019), a number of changes to the programme have occurred. In addition to the ongoing BTM surveillance, three streams of beef industry surveillance have been established, including serological testing of animals entering a beef feedlot, serological testing of samples from animals collected at the time of routine TB testing, and serological testing of samples collected at slaughter. Additionally, some of the previously signalled changes in data handling, aggregation and reporting have been implemented.

The recent identification of a cluster of infected herds in the Canterbury region, initially via bulk tank milk testing and subsequently by tracing, has illustrated that previously unknown infections are still likely to be present, but that with enhancement of operational systems, such clusters can be rapidly identified and controlled. It is expected that additional infected herds may be identified over time as a result of ongoing casing, tracing, and background surveillance.

While the rate of detection of new cases is declining, suggesting a reduced rate of spread of infection, it is unlikely that all infected herds have been identified, or that onward spread has completely ceased. Hence the programme is still within the “delimiting” phase and further work is required before the programme can move into provisional freedom from infection status. Additionally, depopulation of all known infected properties will be required prior to declaration of provisional freedom from infection.

4 Review process

The TAG was provided with three specific terms of reference (TOR; listed below) and with a number of documents by MPI. Additionally, presentations were provided by key staff within the programme, and staff provided responses to specific queries. Finally, MPI and industry partners (DairyNZ and Beef and Lamb) provided feedback, on a draft report within the context of the TOR, to the TAG.

The TAG reviewed the documents provided, the oral presentations, responses by individuals within the programme and representatives from the partner organisations to questions put by the TAG and consulted relevant scientific literature.

It should be noted that no assessment of the operational capability, functionality or efficiency of the programme was undertaken, as it was beyond scope of this review to comment on these matters.

5 First term of reference

*"Review technical aspects of current control and surveillance processes that are related to **delimiting surveillance** for their utility to the current phase of the programme."*

The TAG interprets 'delimiting surveillance' as meaning surveillance to find herds with *M. bovis* infection based on networks of known case herds, that is 'casing and tracing' (both trace back and trace forward).

The TAG commends the programme for the improvements in the management of herds once they have been detected, including tracing and casing, time from detection to movement control, time herds are under movement control before they are confirmed free of infection, and management of herds that are confirmed to be infected. These improvements contribute to all three objectives of the *M. bovis* programme.

Improvements in tracing and casing include improved algorithms for interrogating the NAIT database with the effect of more rapid and more complete identification of animal movements associated with infected herds, and improved approaches to eliciting information from farmers about their social and contact networks, which may identify movements of animals not recorded in the NAIT database. Furthermore, there have been improvements in completeness and timeliness of recording of movements in NAIT. Closer collaboration with OSPRI has enhanced data transfer. Currently NAIT has limited options to define farm types (dairy, beef, dry, breeding), and the ability to only record two production types; currently the majority of properties are simply classified as one of dairy or beef. Additionally, farm types are not always recorded accurately by the farmer, farms can have multiple enterprise types, and the types of enterprises may change within farms over time. The lack of detail within the NAIT system about production type remains a limitation both for tracing and for epidemiological understanding of transmission of infection between herds. For example, multiple classes of stock or production types on one farm are generally not captured and maintaining up-to-date records of production type(s) is challenging, given the dynamic nature of the non-dairy sector. Enhancing two-way flow of information from NAIT to the programme and *vice versa* would improve data quality in both databases. The programme's Tiaki database should have additional fields added to capture additional information (e.g., additional enterprise types), but with continued emphasis on keeping data up to date and accurate.

Incomplete and/or inaccurate animal movement records in NAIT or in EDIR reports result in potentially undetected transmission of infection. Discovery of undetected

infection is being addressed by background surveillance (BTM testing and non-dairy sector surveillance) and via the proposed retrospective reassessment of selected infected herds identified earlier in the programme (the 'Network Assurance Project' (see 5.2)). Identification of infected dairy herds in Northland and the South Island West Coast, and a recent cluster in Ashburton, demonstrates the value of background surveillance and enhanced use of information collected on farm in finding herds not detected by network surveillance.

Improvements in operational performance have been demonstrated by reductions in the time interval from detection to casing to application of a notice of direction (NOD). Modelling (provided as a draft paper submitted to Transboundary and Emerging Diseases by Jordan et al.) has demonstrated that network surveillance has reduced the time from infection to detection and the time from detection to implementation of movement control, with an associated reduction in the risk of onward spread of infection. It is noted that the interval from infection to movement control averages about 6 months, but in some cases may be several years. Tracing windows have been based the recorded likely infection date; hence the TAG acknowledges that the programme has correctly taken a conservative approach when the actual date of infection for a property is not known (i.e., these are assigned an infection date of 2015), and that the earliest of possible date is used where multiple dates are feasible to ensure that tracing includes the likely infectious period. However, long intervals between infection and detection are apparent even where properties assigned an infection date of 2015 are excluded from analyses.

The estimated dissemination ratio has also declined and has been less than 1 since early 2018. However, there are a number of caveats around this modelling, including the fact that the data used for that specific analysis are now more than 12 months old (i.e., August 2017 - September 2019). Implementation of the beef surveillance systems has occurred since this analysis was undertaken, likely increasing the sensitivity of the overall surveillance system. More recent data from the quarterly governance reports continues to support an R_0 and EDR of less than 1.

The TAG suggests that inclusion of genomic data (clade and subclade) within this modelling may help clarify which of the multiple potential source herds was most likely to have been the infection source. The current model excludes cases with multiple sources ('parents'), as currently tracing data may be unable to define the parent definitively. The TAG also notes that there are very few incident cases included from the period in the first 6 months of 2019, the period of the so-called "surge", when delays in casing and tracing, and hence in infection to application of a NOD, likely occurred. Hence some care is required in interpreting the interval from time of infection to movement control in this window. Caution is also required in the use of predictive modelling during epidemics, as acknowledged by Jordan et al, but there is value in updating these models on a regular basis. This could provide feedback to governance, management, and the TAG, and so may be a good use of resources. The TAG also note that the results may be biased as they exclude herds

without a known infection date and currently infected but as yet undetected herds, the absence of which would be expected to downwardly bias the observed times from recent infections to detection.

The absence of precise movement data has resulted in an inability to identify a likely infection date for 12 of the 13 dairy herds detected through bulk tank milk surveillance in 2019 and 2020. The median time from infection to detection in Dry Beef herds was approximately 1.5 years for herds detected in 2019 and 2020. Identifying why detection took so long could improve the surveillance systems.

The TAG notes that ‘non-dairy’ enterprise types were the recorded sources of infection for 5 dairy farms (involving 7 dairy herds) identified during 2018 - 2020. Four of the five relate to co-grazing of dairy heifers from multiple farms during grazing on dry stock farms, while the likely route of infection in the final case was movement of beef bulls used for dairy breeding purposes. These data indicate the potential for transmission from ‘non-dairy’ to dairy herds, and transmission between dairy stock from different farms while co-grazing away from their ‘home farm’. Details of these risk pathways for infection of dairy herds while on non-dairy properties are discussed later. It is also noted that lack of clarity about enterprise type in databases means assessing the risks of transmission between various enterprise types is difficult.

5.1 Canterbury cluster (2020)

A cluster of 9 infected herds (including 5 dairy herds) has recently been identified in Canterbury, with 4 of the 5 dairy herds detected through BTM testing in August-September 2020 and a subsequent 5 herds detected through network surveillance. Another possibly unrelated infected dairy herd was recently detected via BTM testing in the Selwyn district of Canterbury.

It is encouraging to see that the BTM surveillance continues to find infections. It was not unexpected to find further cases of *M. bovis*, especially in the Ashburton area as this has had the highest density of infected herds in the country. Following detection of BTM positives, the programme was able to rapidly identify high-risk contact herds. The improvements in the rate of integration of the epidemiological and genomic data to help identify and confirm transmission routes is a positive development that the TAG strongly commends. This cluster appears confined to the Ashburton area and the isolates sequenced from herds thus far are *M. bovis* NZ clade 2. The local clustering of infection of the same clade demonstrates that there are not multiple herds within the same geographical area infected with unrelated clades. Had this been the situation, it might have indicated multiple unrelated sources of *M. bovis*. Additional isolates are to be sequenced, providing more detailed genomic information.

However, the source of this cluster is currently unknown and further casing, epidemiological investigations and genomic analyses are needed to identify this. In addition, this cluster reinforces the challenges to *M. bovis* eradication due to the extensive movements of cattle that are not recorded in NAIT.

Taking into account the available biological, epidemiological and genomic evidence, it is the view of the TAG that the presence of a large feedlot in the Canterbury area is a plausible source of infection for the current Canterbury cluster. This feedlot has been infected for a number of years. There are dairy cows within 500 m of the boundary of this property and the *M. bovis* isolates from the two herds in the cluster sequenced before December 2020 were the same sub-clade type and were very closely related to isolates from the feedlot. Alternative sources for this cluster include unrecorded movements of animals from a presently unknown infected property or properties. It is the view of the TAG that the possibility that this feedlot is the source of infection should be examined closely, including assessment of current biosecurity measures, use of sentinel animals, and ongoing monitoring of properties on the boundary of this feedlot. The current management plan should be reviewed to ensure that any risk of transmission is minimised. The TAG is aware that the programme is actively assessing potential risks associated with the feedlot and strongly supports these efforts. At some time point, prior to declaration of provisional freedom of disease, depopulation of this property will need to occur. The TAG recognises that this will be a complex operation with significant logistical and financial implications. It is important that this process is carefully planned, and the timing will need to weigh the potential risk that the feedlot is a potential source of infection for other properties against the risk of possible reinfection of the feedlot after depopulation and re-stocking.

5.2 Network Assurance Project

The *M. bovis* programme has proposed a “Network Assurance Project” with the aim of identifying infected herds that were not identified through previous network surveillance.

The project will undertake retrospective investigations of a selection of high-risk case herds in the 2018 and 2019 calendar years, applying the improved algorithms to assess NAIT data and improved approaches to farmer interviews to elicit information regarding social networks through which animals may have been moved but not recorded in NAIT. It will also integrate genomic sequence data with movement data to help define sources and time of infection more accurately for herds where previous estimates were not clear.

Finding no further infected herds through the Network Assurance Project would provide additional assurance that there is no residual undetected infection, supporting the transition from the delimiting to the provisional freedom from infection phase.

There are several underlying assumptions which must be met if the Network Assurance Project is to be beneficial. These include the availability of data from either NAIT or farmer recall to identify previously unidentified movements. There is concern that some farmers may not wish to revisit past interactions with MPI. Given the significant time that has elapsed, there are real issues related to the validity of farmer recall about details of movements of animals and other risk items.

The Network Assurance Project is likely to provide additional assurance that network surveillance has been effective and that few residual infected herds remain and hence that the end of the delimiting phase has been reached. This will provide further confidence for the move to the provisional freedom from infection phase. The TAG supports the Network Assurance Project but suggests that a clear stop/go point is established for this project such as when “x” high risk herds have been re-examined with no further infections detected.

5.3 Genomic information

The ongoing genomic sequence analyses have continued to support the inference that there was a single introduction of *M. bovis* into New Zealand around 2015. These analyses also provide confidence that all recent cases for which a sequence is available have a close linkage with previously identified infected herds. The currently infected properties that have had isolates sequenced have very closely related genotypes within a single clade. The genomic sequencing and analysis is now being conducted relatively soon after detection of infected herds and thus can be expected to play an increasingly important role, along with contact tracing, in determining the most likely transmission pathways. As this analysis can predict the most likely relationships between isolates from multiple infected farms, it will not only confirm inferences from tracing data, but also identify gaps in tracing data that can result in misleading inferences about transmission pathways. Furthermore, it will provide ongoing assurance that there are no infected networks that have not been detected previously by surveillance or contact tracing. This will be of increasingly importance when or if sporadic cases are detected over the next few years.

The programme currently has at least one whole genome sequence for 104 of 260 case farms (40%). These are weighted towards farms that were detected in 2019 and 2020, as few sequences are available from farms identified in 2017 and 2018. Where PCR-positive samples from farms from which no sequence data are available have been retained, efforts are being made to obtain an isolate and hence a sequence type to further the understanding of the epidemiology and transmission of *M. bovis*.

5.4 Key performance indicators for detection and management of infected herds

To understand progress of the programme and hence to comment on the utility of the current processes, both operational and disease focused metrics are required. The integration of the customer relationship management, laboratory information management, NAIT, and other data streams into a central data depository (ADS) is a substantial enhancement of the programme. The provision of “operational” management reports and governance reports (e.g., the governance dashboard) reflects the improvements in the data reporting capability of the programme. While understanding that there are data validity and privacy concerns to be addressed, the TAG strongly recommends that automation of the key epidemiological reports be fast tracked as they remain essential to understanding the epidemiology and hence support surveillance decision-making. Greater detail about infection pathways and times (for example, the time interval, the median and range of time intervals from infection to detection of daughter herds, and the number of movements off herds between likely infection and detection, and the proportion of those forward movements resulting in subsequent infection) are all critical metrics to enable assessment of the programme.

Having clear and useful metrics, including number of animals and enterprises tested, during the provisional freedom of infection phase will be important to monitor progress and for communications to stakeholders. It is likely that more cases will be detected over time, but communication about the amount of testing and the proportion of tests that are test negative will be important to reassure stakeholders that there is ongoing activity, and that confidence of likely freedom from infection is increasing with time. Maintaining stakeholder engagement over a period of up to 10 years, when the perception may be that little is happening other than ongoing ‘background’ surveillance, may be challenging for the programme.

5.5 Laboratory capability and quality assurance systems

The combination of the AHL and other commercial laboratories appears to have sufficient capability to meet the needs of both the delimitation phase and for ongoing background surveillance. The TAG understands that the current arrangements with AHL are in place until June 2021, and that a Request for Proposals is underway to support beef sector surveillance.

The TAG recommends that laboratory requirements for surveillance needed through the provisional freedom of disease phase be clearly defined and appropriate laboratory contracts be put in place to ensure that the programme’s needs are met. Whether such testing is undertaken by AHL or commercial providers is not critical, so long as robust oversight of testing with appropriate quality assurance systems are in place.

Current quality assurance processes appear robust, with all laboratories meeting IANZ assurance standards. Splitting selected samples and testing them simultaneously in different laboratories would provide greater quality assurance than the existing approach of distribution of samples for proficiency testing, as proficiency test samples are more likely to receive special attention in a laboratory and thus are less likely to reflect typical laboratory results.

6 Second term of reference

*"Review technical aspects of the current control and surveillance processes that are related to **scanning and background surveillance** for their utility to the current phase of the programme and for transition to a 'proof of absence' phase."*

Assessment of the utility of the current background surveillance streams is meaningful only in the context of understanding what the overall objectives/goals of the programme are, and in the context of having a clear understanding of how freedom from infection is to be defined. A commentary on the challenges in designing surveillance to demonstrate "freedom of infection" and phases of a programme designed to demonstrate freedom of infection is provided in Appendix 1.

6.1 Non-dairy sector surveillance

6.1.1 Why non-dairy sector surveillance is required

The non-dairy sector is heterogeneous as it includes both beef enterprises (breeding, finishing), as well as enterprises that are rearing calves and/or dairy heifers for return into the dairy industry. This is further complicated by the fact that these enterprises may have multiple different livestock classes present.

The risks of infection associated with these different enterprise classes are difficult to define, in part because the enterprise type has been poorly defined in NAIT and thus in the MPI database.

As a result it is difficult to provide definitive commentary on the effectiveness of the surveillance system, which requires clear definitions of the risks of transmission and the prevalence of infection and the incidence of infection amongst the different non-dairy enterprise classes.

This does not necessarily mean that the prevalence and incidence of infection in the non-dairy sector is likely to be high, but rather that there is some uncertainty about this. Given the probable low prevalence and incidence amongst these enterprises, the surveillance program for them needs to have a low design prevalence. Thus the TAG recommends that the surveillance system is designed in such a way that it accounts for the likely lower design prevalence to ensure sufficient sample size (both at herd and within herd level) to provide a high degree of surety that the prevalence is indeed low amongst these enterprises.

Bulk tank milk surveillance is providing information about *M. bovis* in lactating dairy cow groups but there remains uncertainty about the prevalence of *M. bovis* in other animal classes, groups and herds, including dry dairy cow and dairy replacement heifer mobs, beef breeding herds, and calf rearing, beef finishing, contract grazing and other enterprises sourcing animals from the dairy industry. These categories are apparently referred to as “beef” or “other” in the MPI situation reports. However, as the type, source, and fate of animals on these properties is not clear, the use of the term “beef” is simplistic and does not reflect the range of cattle production activities falling under that heading. This is important because *M. bovis* prevalence, incidence and risk of forward transmission will likely vary with production activities. Similarly, herds classified as “other” could be using a wide range of production activities. We will refer to lactating dairy cow groups as “dairy” and all others as “non-dairy” animals, groups and herds throughout the rest of this report, but at times differentiate between non-lactating animals of dairy origin and beef animals. There are limited data on the risks of *M. bovis* transmission between various types of non-dairy herds, and from lactating dairy herds into the non-dairy sector and *vice versa*. This lack of clarity about enterprise type means assessing the risks of transmission between various enterprise types remains difficult.

The key issue from a surveillance perspective is availability of monthly bulk tank milk ELISA data for lactating dairy herds but reliance on blood sampling of individual animals in the non-dairy sector. This presents significant logistical challenges. However, as for the dairy sector, for national freedom, not only must any remaining *M. bovis* in the non-dairy sector be identified and removed, but there must be sufficient surveillance across the various enterprise types in the non-dairy sector to provide a high degree of confidence of freedom from infection. Given the likely low animal-level prevalences within infected non-dairy herds, considerable numbers of animals must be tested in a substantial number of herds to gain high confidence of freedom.

There is extensive movement of cattle between different enterprise types. For example, dairy-beef animals transferred from dairy to non-dairy herds, grazing of replacement dairy heifers away from milking farms, off-farm dairy herd winter grazing arrangements and service bulls moving to dairy farms. Over half of the infected herds detected to date are recorded as ‘beef’ herds in MPI statistics with a further 25% recorded as ‘other’ and less than 23% recorded as ‘dairy’ herds. However, as discussed above, the TAG is unclear about how many of these infected ‘beef’ herds are defined as such due to infection being detected in animals of dairy origin. In addition, the forward risk of transmission from these non-dairy enterprises to both other non-dairy enterprises and back to the dairy industry remain unclear.

While the majority of dairy animals entering the non-dairy sector are destined for slaughter, there are a significant number of animals moving from non-dairy to dairy farms (e.g., dairy heifers and beef bulls used as sires on dairy farms) and also risks of transmission when dairy heifers and dry dairy cows are grazed on non-dairy

farms. This is particularly the case in enterprises with multiple stock classes, which may not be apparent from the farm's NAIT classification.

Extensive efforts have been made to trace risk movements into and from detected infected herds. However, it is often difficult to identify all risk movements. Furthermore, as within-herd seroprevalence in non-dairy herds is likely to be low, the herd level sensitivity of ELISA testing animals following tracing is not 100%. Thus, it is likely that there are currently some undetected infected non-dairy herds. To declare freedom from infection, it will be necessary to have high confidence that, if any undetected infected non-dairy herds remain, the number of such herds is very few, and that the risk of forward transmission from these herds is low. Ongoing bulk tank milk surveillance may indirectly provide some information about the status of the non-dairy sector as it would be expected to detect infection of lactating dairy herds via dry dairy cows and dairy replacement heifers entering lactating dairy herds including those that have been grazed on non-dairy enterprises, albeit often with a potentially long lag interval between infection and detection.

6.1.2 Challenges in implementing non-dairy surveillance

Challenges in detecting infected herds in the non-dairy sector include the likely low incidence and prevalence of infection at animal level, the low to modest animal-level sensitivity of the ELISA, and the difficulty of sampling animals especially on extensive beef breeding farms.

6.1.3 Non-dairy surveillance streams already implemented

The programme recently implemented the National Beef Cattle Surveillance programme (NBCSP). The aims of this surveillance programme are to:

- Increase the level of confidence that *M. bovis* is not widely disseminated in the non-dairy sector
- Support network surveillance where possible
- Provide broad spatial coverage
- Cover a range of farming systems including commercial terminal beef herds, beef breeding herds, and small and non-commercial farming enterprises.
- Produce and integrate data that can be used to demonstrate that *M. bovis* has been eradicated from New Zealand.

If infected herds are found, these will be managed as part of the *M. bovis* programme response.

The three current streams of the NBCSP are:

- Feedlot Induction Surveillance
- Beef Breeder Surveillance
- Meat Processing Plant Surveillance

Under all three streams, selected animals are blood sampled, and samples tested using an ELISA test.

One additional surveillance system component is currently being planned but is not yet ready to be implemented:

- Small and Non-Commercial Herd Surveillance

6.1.4 TAG's assessment of current non-dairy surveillance

6.1.4.1 General comments

The TAG strongly supports the establishment of the NBCSP. To have established these surveillance streams during the constraints imposed by COVID and the operational difficulties of a diverse non-dairy sector that includes extensive grazing properties and many small mixed model operators is an outstanding achievement. These streams could not have been established without strong relationships between MPI and numerous industry stakeholders.

Major strengths of these surveillance components are the cost efficiencies and minimal additional impositions on feedlot, farm, and meat processing staff, as sampling is being fitted into existing animal handling systems.

The TAG is encouraged by the initial results of this surveillance work which suggest that the herd-level prevalence of *M. bovis* in the subsets of the non-dairy sector surveilled by the NBCSP is low i.e. that *M. bovis* is not likely to be widespread in those subsets of the non-dairy sector. However, these streams by their nature are largely opportunistic, rather than fully representative of the populations being surveyed, and the results do not demonstrate that there are no *M. bovis* infected herds in the non-dairy sector.

In summary, there are potential limitations in these surveillance streams including coverage of herds, the subsets of animals tested, limited numbers of animals tested per herd, possible low incidences and prevalences of infection in infected management groups and herds, and probable low to modest animal-level sensitivity of the ELISA in detecting past exposure. Thus, it is possible that there remain undetected infected non-dairy herds, and there is a risk that true herd-level prevalence of infection in the non-dairy sector is being underestimated by the current sampling strategies. This is not a criticism of progress in the non-dairy sector (on the contrary, TAG strongly applauds the work to date in the sector) but rather simply reflects the challenges intrinsic to this sector.

6.1.4.2 Herds and animals tested

Within the current NBCSP, there is limited ability to preferentially select high-risk herds or animals for sampling. For the Feedlot Induction Surveillance, animals from

one region are overrepresented, although it is acknowledged that there is aggregation of animals on some farms prior to introduction to the feedlot thus increasing the geographic spread of surveillance. This is of benefit to the programme as this is a high risk region, but it also means that that stream provides limited information about herd-level prevalence in other regions. The Beef Breeder Surveillance is currently coupled with TB testing, and so some herds are currently not tested at all because of the low frequency of TB testing in these regions and the herd and animal selection criteria for TB testing. The proposed decoupling of the Beef Breeder Surveillance will allow risk-based herd selection in non-dairy herds under this stream. Similarly, there is limited ability to select for high risk herds to be tested within the Meat Processing Plant Surveillance. Under all three streams, within selected herds, there is also limited ability to preferentially select animals for testing. The TAG recommends risk-based testing of non-dairy herds, targeting regions, herds, animal classes, and age groups at higher risk of *M. bovis* infection. Such risk-based sampling strategies will increase confidence about whether *M. bovis* is present or absent in non-dairy populations.

6.1.4.3 Assumed animal-level prevalence of infection within infected non-dairy herds

There is little knowledge about the infection dynamics of *M. bovis* in various types of non-dairy herds, and the TAG understands that analyses of past surveillance and slaughter test results to estimate within-herd prevalence of infection would be a large task and unlikely to yield useful results, due to historic non-random sampling of trace herds, the small numbers of herds involved, and inability to define animal management groups within herds. Given the more extensive nature of many non-dairy herds, it is likely that the rate of spread within such herds is slower than in dairy herds. In addition, not all management groups on a farm may be infected which would result in low prevalences of infection if data from multiple management groups were pooled.

An Australian study found the prevalence of seropositive animals ranged from 5% in several feedlots to 30% in one feedlot when young beef breed animals were sampled when entering feedlots from grazing properties. However, given the uncertainty about transmission rates within infected non-dairy herds, it seems prudent to assume low frequencies of transmission, and hence low prevalences of infection within infected non-dairy herds. Low prevalence of infection results in difficulties in detection, but conversely reduces risk of transmission to other groups and potentially increases the probability of spontaneous elimination of infection from the herd (e.g., if infected management groups are sold for slaughter without first transmitting to other groups on the farm).

6.1.4.4 Assumed animal-level diagnostic sensitivity of the ELISA

ELISA results from serum of individual animals are interpreted at herd level. However, herd level sensitivity is determined, in part, by animal-level diagnostic sensitivity and specificity of the ELISA. For PCR-positive cattle in the *M. bovis* programme, 79% were ELISA positive at an S/P ratio threshold of 90 (Mackereth and Marquetoux, 2019), indicating quite high but imperfect diagnostic sensitivity for detecting currently infected animals. However, animal level diagnostic sensitivity for detecting past exposure to *M. bovis* declines over time following exposure. Amongst initially seropositive cattle, the proportion that are seropositive decreases over time, and seroprevalence of groups also declines with time amongst calves and cows in infected herds. In an Australian study (Hazelton et al. 2020), the seroprevalence of calves from known infected herds declined markedly over time and about two thirds of the animals that were seropositive at weaning were no longer seropositive pre-breeding (i.e., about 9 months later). One of the 356 heifers in that study was culture-positive following calving, demonstrating that heifers may act as carriers and potentially (re)infect dairy herds. The ELISA used in that study was probably inferior to that in use in the *M. bovis* programme, but these results illustrate that the median duration of seropositivity from first exposure may be less than 9 months. Similarly, a Finnish study demonstrated that seroprevalence declined with time amongst calves and cows in herds confirmed to have *M. bovis* based on PCR or culture (Vähänikkilä et al. 2019). It is not known whether the decline in seropositivity is due to infected cattle eliminating infection and becoming seronegative and/or whether seroprevalence declines even in cattle that remain infected (i.e., whether the sensitivity of the ELISA declines with time since infection). However, regardless of which of these occurs, these studies demonstrate that titres decline over time making infected herds more difficult to detect.

6.1.4.5 Assumed animal-level diagnostic specificity of the ELISA

Using animals deemed uninfected, the animal-level diagnostic specificity of the ELISA was estimated as 98% at an S/P ratio threshold of 90 (Mackereth and Marquetoux, 2019). However, from the document provided to the TAG entitled ‘Overview of the National Beef Cattle Surveillance programme 8 Nov 2020’, of 80,557 blood samples collected under that programme, 52 were ELISA positive. If all 52 were false positives (i.e., none were infected with *M. bovis*), the diagnostic specificity of the ELISA would be:

1-(52/80,557)=0.999354494 or 99.94%

If any of those 52 ELISA positive animals were infected with *M. bovis*, the diagnostic specificity of the ELISA would be even higher than this.

The difference between these specificity estimates may be in part because some cattle acquire infection and seroconvert, then spontaneously clear infection (and hence are PCR negative) but remain seropositive for some time. As non-dairy herd

surveillance is based on the ELISA at animal level, lower animal-level diagnostic specificity of the ELISA due to such animals in infected herds will increase herd level sensitivity, all else being equal.

The likely higher than previously estimated ELISA specificity alleviates concerns about excessive numbers of false positive herds and the resources required to ascertain the true infection status of such herds. Strategies to minimise numbers of false positive herds were, completely soundly, recommended to MPI assuming the animal-level diagnostic specificity of the ELISA was 98%. But the very high specificity demonstrated from the recent results shows that such strategies are not required. This simplifies the surveillance design and avoids reductions in herd level sensitivity that such strategies may have caused.

6.1.4.6 Seroprevalences in infected non-dairy herds

Seroprevalence in infected herds is determined by the three parameters discussed above (assumed animal-level prevalence of infection and assumed ELISA animal-level sensitivity and specificity). Thus seroprevalences observed in network surveillance testing in non-dairy herds can provide understanding of the values of these parameters. The TAG recommends that seroprevalences from network surveillance testing in non-dairy herds be fully reviewed, to inform the selection of values for these three parameters for use in scenario tree modelling and other modelling to support freedom from infection. This review should consider seroprevalences from non-dairy herds subsequently deemed uninfected along with those subsequently deemed infected. The methods of detection of these herds should be carefully considered when extrapolating these seroprevalences to as yet unidentified infected non-dairy herds, to ensure there is no bias due to the methods of detection in the observed infected herds relative to seroprevalences expected in as yet unidentified infected non-dairy herds.

6.1.4.7 Quantitative assessments of herd level sensitivities

Herd level sensitivities decline with small numbers of animals tested in each herd, as is common under the NBCSP. Effects of various test sensitivities, specificities and interpretation on herd-level sensitivity at low within-herd design prevalences have been assessed in Appendix 2.

Summary

The herd level prevalence of *M. bovis* in non-dairy herds is probably low in New Zealand. There are considerable challenges in actually demonstrating this with a high degree of confidence, and given the possibly serious consequences of declaring national freedom before all infection has been removed, the TAG recommends that, when doing scenario tree modelling and other modelling to support freedom from infection, unless there is good evidence to the contrary: a) animal-level design prevalence of infection within infected herds should be set to a

low value, b) only modest animal-level diagnostic sensitivity of the ELISA for detecting infection be assumed, c) quite high animal-level diagnostic specificity of the ELISA be assumed, and (d) that detailed investigation of herds with small numbers tested and only one test positive be undertaken (in addition to the current approach of detailed investigation of herds with two or more test positive animals). Values for these parameters should be jointly chosen such that expected seroprevalences based on these parameters are at the low end of seroprevalences observed in infected non-dairy herds to date.

6.1.5 Building on the existing non-dairy surveillance programme

The TAG recommends additions to the existing non-dairy surveillance programme:

(1) As recommended by Ausvet (Cowled et al. 2020), further scenario tree modelling should be conducted to inform how long it would take to achieve high confidence of freedom from infection under various assumptions and scenarios. As discussed above, that modelling should assume very low seroprevalences in infected herds and should assess the sensitivity of results to conservative assumptions for other key parameters, including probabilities of transmission such that within-herd prevalences in infected herds and herd-level prevalences do not increase over time. If there is strong evidence that there is no infection in the non-dairy sector, a low starting prior probability of infection in that sector may be appropriate but the basis for this should be explicitly documented and that evidence submitted to detailed critical review.
(2) Assess the impacts of testing more cattle per herd at each herd test and testing more herds each year in the non-dairy sector. As these strategies would increase programme costs and logistical demands on the non-dairy sector, the likely benefits and costs must be fully assessed for both the programme and the industry participants. Again, modelling would be useful for such assessments. In relation to testing more cattle per herd at each herd test, note that given that the majority of non-dairy herds have relatively low numbers of cattle, herd-level sensitivity for detecting herds with only 1 or 2 infected animals will not be high even if all animals in the herd are sampled, as herd-level sensitivity in that situation is limited by the animal-level sensitivity of the ELISA. The increase in animal-level and hence herd-level sensitivity if ELISA interpretation is changed from SP ratio cutpoint of 90 to 60 can also be assessed as part of this, using animal-level sensitivity
(3) Conduct a detailed risk assessment of animal movements between non-dairy herds, and between non-dairy and dairy herds, in combination with detailed assessment of opportunities for transmission (both within and between management groups) on non-dairy farms. It may be useful to expand this work to include some transmission modelling (both within and between management groups) based on those data.

    The aims of this work should be:

i) to assess risks of *M. bovis* entry to, and transmission from, non-dairy herds with various production systems and combinations of production systems,

ii) identify higher risk herds i.e., herds at greater risk of being/becoming infected and with greater risk of transmission to other herds, and

iii) to detail herd production systems including complexities such as multiple production systems and patterns of change in these over time.

This work should be designed to provide information on key questions such as the following:

1. What is the risk of transmission via:
    a. Bulls
    b. Dairy breed calves from calf rearers
    c. Dairy heifers grazing on farms with non-dairy herds
    d. Dry dairy cows grazing on farms with non-dairy herds
2. Are the herd-level prevalences and within-herd prevalences in infected non-dairy herds likely to be stable or even increase over time? Is infection ever entirely removed from non-dairy herds when mobs of animals are sold for slaughter?
3. What more refined methods are required for categorising and recording herds with multiple concurrent production systems? What is a practical method for continually updating centrally recorded production systems for herds?

This work should use between-herd movement data collected for case herds (both data already collected, and any further data collected under the Network Assurance Project, and including transmission patterns in the current cluster), NAIT data, and prospectively collected data from selected uninfected herds.

(4) As an adjunct to item 3, assess in detail the empirical evidence from existing data of transmissions from non-dairy to dairy herds to date using all relevant case herds.

The TAG understands that, for a number of the infected dairy herds, the source of infection was attributed to a non-dairy source. In addition, for quite a number of infected non-dairy herds, the source was attributed to another non-dairy herd. The TAG recommends a detailed reinvestigation of how these herds became infected, and greater clarity sought as to the true nature of the enterprises involved. The TAG acknowledges that the risks are likely to be different between, for example, closed beef studs compared with large calf rearing operations sourcing animals from multiple dairy sources.

This should include assessing whether whole genome sequencing data support the attributed source, along with allocating semi-quantitative probabilities to the various possible sources for each herd. This may involve the source herd not necessarily being the physical farm where transmission probably occurred. For example, if cattle from dairy herd B mixed with, and probably contracted *M. bovis* from, cattle from dairy herd A while on a dry beef farm (Herd C) but had no contact with Herd C cattle, the most likely source for Herd B would be recorded as Herd A and not Herd C. At the same time, Herd C would be identified as a high-risk farm enterprise, which could be targeted for risk-based surveillance of animals moving off the farm. This investigation may help elaborate risk pathways from non-dairy herds.

TAG recommends data summaries for case herds be expanded to include the following fields:

- Herd clade
- Probable source herd ID
- Source herd clade
- Next most likely source herd(s)
- Next most likely source herd clade(s)

The TAG understands that the date of infection is chosen 'to be conservative' where the source of infection is unknown, i.e., the date chosen is defaulted to 2015. This is done to ensure the tracing includes all relevant movements (i.e., erring on the side of a wide tracing window over an unduly narrow tracing window that may have missed some important movements). While this make sense for selecting movements for tracing, the TAG suggests that a new field be created and populated to store tracing window start date (a field for management purposes), and the source herd fields we have suggested above be used solely for epidemiological purposes (probable source herd ID field, next most likely source herd(s) fields etc).

Items 3 and 4 should be designed to build on the *M. bovis* epidemiology risk prioritisation work already conducted. Some features of the additional recommended work are:

- Use of genomic data
- Better herd production system categorisation accounting for mixed systems, and changing systems over time
- More nuanced assessment of possible sources to case herds

(5) Implement more risk-based surveillance

The TAG strongly endorses the proposal to add risk-based surveillance for the non-dairy sector to the NBCSP. Sampling of animals in conjunction with

TB testing has been a relatively easy way to implement surveillance of this sector but has some biases. Testing of herds independent of TB testing will allow targeted selection of non-dairy herds by risk and ensure coverage across all important non-dairy production systems. It is recommended that risk-based surveillance include:

1. increased testing (higher number of herds and higher number of animals within herds) in higher risk regions, and
2. focus testing on higher risk herds (i.e., herds at greater risk of being/becoming infected and with greater risk of transmission to other herds) within regions.

Thus, the detailed work described under items 3 and 4 above will be essential to inform the design of risk-based surveillance streams, along with other considerations. For example, it is likely that the risk of infection in non-dairy herds is higher in areas where more infected dairy herds have been found.

Analysis of infected non-dairy herds detected in 2019 and 2020 show the highest proportion were in Canterbury and Northland. This aligns with the areas with more infected dairy herds. Also, of dairy herds detected through bulk tank milk surveillance in 2019 and 2020, a relatively high proportion were located on the South Island West Coast, which may indicate that this is another area with a possibly higher risk of undetected infection in non-dairy herds.

TAG considers that risk-based surveillance of non-dairy herds is preferable to a representative sampling survey. Provided risk factors are correctly identified and an appropriate design is used, risk-based surveillance would be expected to result in greater confidence of freedom for the same cost (or the same degree of confidence of freedom for less cost) than a survey using representative sampling.

(6) At this point in the programme, following monthly testing of all dairy farms for over 18 months, the non-dairy sector may take on a relatively greater importance as a potential source of remaining infections. At the same time it is clear that the challenges in finding any infected non-dairy herds is going to be difficult and time consuming. Consideration should be given to ways to break transmission between the two sectors through changing the behaviours of farmers by raising their awareness of the associated risks. Continued messaging is required around the risks in off farm grazing and other opportunities for mixing of cattle, whatever the source of those cattle.

The TAG's key message for strategies relating to the non-dairy sector is that additional work is required to ensure eventual high confidence that the number of infected herds is very few. While there may truly be relatively few undetected infected non-dairy herds, and a relatively low risk of non-dairy to dairy transmission,

the existing evidence is not sufficient to reach these conclusions with a high level of confidence. The TAG notes the low test positive rate amongst the over 80,000 animals from nearly 4,000 herds tested when the TAG was last provided with updated beef surveillance information in November 2020. It is the TAG's understanding that significant additional testing has occurred in the interim, and assuming that the incidence of confirmed infection remains low, provides greater surety about a low prevalence within the non-dairy sectors.

6.2 Dairy heifers

It is common management practice in New Zealand that replacement dairy females ('heifers') are managed remotely from the farm of origin and returned as pregnant animals a month or two prior to 1st calving. Potentially these replacement animals could become infected via colostrum or milk on an infected dairy farm, never show clinical signs, and hence remain undetected until they calve approximately 2 years later and contribute to the bulk tank which is then tested by ELISA. There is also potential for them to become infected via contact with infected stock from another farm (dairy or non-dairy), as some contract grazing farms do not separate animals from different source farms.

Thus, under the current background surveillance system focused on testing of bulk tank milk, there may be a lag of up to two years between infection to detection (i.e., infected heifers may be detected only after calving and entering a lactating herd), and an associated risk of transmission to other groups of animals in the interim.

Relatively few of these animals have been bled. These animals have not been targeted as part of the sampling associated with TB testing. As outlined above, as the TB testing is focused on high risk TB herds rather than high risk *M. bovis* herds. The TAG understands that a request for proposals to do risk-based surveillance of dairy heifers independent of the TB testing programme is underway. Negating some of this concern, where the source of infection for dairy replacement animals is milk from a dairy herd (whether the herd in which they were born or another herd), that herd will be monitored through bulk tank milk, and this would be expected to markedly decrease the interval from infection to detection in infected dairy heifer groups.

The potential benefits of undertaking an additional surveillance stream for dairy heifers should be evaluated. Earlier identification of infection in dairy heifers will likely reduce the possibility of onward transmission to other groups of animals on non-dairy farms and of dairy herds. Use of a risk assessment and scenario tree modelling would enable assessment of the biological and economic benefits (or otherwise) of this additional surveillance stream. Assumptions about prevalence of infection in infected management groups and animal-level diagnostic sensitivity of the ELISA should be guided by the discussion above under 'Non-dairy/beef sector surveillance'. As for non-dairy herds, TAG recommends that a low animal-level design prevalence

of infection in infected groups and a relatively low animal-level diagnostic sensitivity be used and included in sensitivity testing within such modelling for dairy heifers.

6.3 Bulk tank milk surveillance

The rapid establishment, refinement, and ongoing implementation of the bulk tank milk surveillance programme is one of the major achievements of the eradication programme. Some infected herds have been first detected through bulk milk testing. While some of these herds may have been subsequently detected through other surveillance methods, the bulk tank milk surveillance programme likely resulted in earlier detection than would otherwise have been the case. Equally importantly, the negative results from the bulk tank milk surveillance programme are providing crucial information that the current number of undetected infected lactating dairy herds is probably quite low.

The ELISA is highly specific (based on the very low proportion of herds that test positive in each round) so the number of herds testing positive and requiring investigation is manageable and not consuming excessive amounts of resource. The fact that most test positive herds are found on investigation to not be infected is expected when the herd-level prevalence of infection is very low and does not indicate that the specificity of the ELISA is low.

The question has arisen of whether to reduce the number of months when bulk tank milk testing is conducted. There is a distinct seasonality in test positives on the bulk milk screening testing, with the majority of herd test positives occurring in early spring, and late autumn. Biologically this is plausible as the majority of dairy herds in New Zealand are seasonal (spring) calving. The spring peak may be associated with infected animals calving first time (i.e., heifers returning from grazing) or recrudescence of infection associated with the stress of transition to lactation. Autumn detections may be due to recent infections (for example due to introduction of infected bulls in the latter part of the seasonal breeding programmes) or associated changes to the mammary gland near the end of lactation. Alternatively, the peak in detections autumn may be associated with those herds that calve in autumn. Hence temporarily focused sampling around early and late lactation may improve the cost effectiveness of the programme. However, this approach could only be implemented if data on either commencement date of supply or date of start of calving were available to allow targeted sampling and if the cost savings of such an approach without any loss of sensitivity.

One of the strengths of the bulk tank milk surveillance programme is that each testing round is close to a census of all herds supplying milk to a milk processor. However, owners of dairy cows not supplying one of the milk processors (e.g., those selling raw milk or artisanal cheese products) may not be captured in the current surveillance system. While the number of herds involved and the potential risk of infection and forward transmission from these herds may be low, a risk assessment of these herds should be undertaken.

6.4 Risk assessment of potential transmission pathways

There are a number of residual risks to the success of the *M. bovis* programme. Examples of these residual risks include:

- any small scale dairy producers who may fall outside of the bulk tank milk surveillance,
- herds where imported animals reside or have resided in the recent past, and
- dairy service bull providers.

The TAG recommends that the programme consider formal risk assessment for certain niche production groups to provide a basis as to whether they constitute a programme risk and whether they should be included in surveillance.

7 Third term of reference

"Review the construction and use of existing scenario tree models (STM) to optimise the beef and dairy surveillance programmes (note the STM has economic components). Suggest supplementary modelling approaches if required.

7.1 Definition of national proof of freedom from infection from *M. bovis*

A definition of "disease absence" has been provided in programme documents. Additionally, it is proposed that disease status be reported using OIE guidelines. It is important that there is widespread agreement on the specific programme endpoint, and an explicit statement defining the concept of national freedom and broadly how this will be achieved is required. Establishing clear milestones to be achieved prior to declaration of provisional freedom of infection is very important, as it will inform stakeholders and the farming community more generally. The TAG considers that specific definitions of phases leading to such a declaration of "freedom from infection" should be developed, and that detailed planning of operations and communications during each of the phases should be undertaken. This planning should include criteria for declaring when each milestone is achieved. These issues are discussed further in Appendix 1.

Our understandings about the programme endpoint of national freedom are that:

1. the concept of national freedom from *M. bovis* means no *M. bovis* is in New Zealand,
2. this will be achieved by removing *M. bovis* from the domestic cattle population in New Zealand, and
3. when 2) is achieved, by inference based on other knowledge, it will be assumed that *M. bovis* is not present in other species (including those listed

below) or in the environment in New Zealand (or, if present in any of these, there is negligible or no risk of transmission to domestic cattle)

a. Other domestic species including small ruminants
b. Bison
c. Asian water buffalo
d. Zoo species
e. Wildlife
f. Environment

7.2 Role of Scenario Tree Models

The programme has commissioned development of a scenario tree model (STM) by Ausvet and it is the TAG's understanding that these models will be used to help inform decisions about the mix of surveillance strategies required as the programme moves towards provisional and final freedom from infection.

The TAG concludes that scenario tree modelling is a recognised and valid technique to support the planning of surveillance to demonstrate freedom from *M. bovis* in New Zealand. Scenario tree modelling can legitimately be used to assess various surveillance approaches and to analyse the collected data.

The TAG was provided with a report prepared for the programme on scenario tree modelling to support *M. bovis* surveillance by Ausvet (Cowled et al. 2020a). An addendum to this report (Cowled et al 2020b) presented financial assessment of the previously identified scenarios and made recommendations for further work. Although these reports include recommendations for changes in surveillance strategies, the TAG understands that a major objective of this work was to introduce programme to use of the method.

Ausvet collaborated with the programme during a training workshop (11–14 November 2019) to understand the outbreak and surveillance system and to plan and parameterise an appropriate scenario tree model. The model was implemented in R and was provided with instructions for further use by programme staff. Simulations and sensitivity analyses were conducted, and inferences made about future freedom surveillance approaches.

During meetings with MPI staff, TAG was advised that MPI staff have the capacity to run and modify the model, although to date it has not been widely used.

Risk of infection to the population is based on sector (dairy versus non-dairy), region, herd size and herd type. This allows for simulation of data for risk-based sampling, e.g., preferential testing larger herds in high risk regions as they are more likely to be infected.

The following surveillance streams were included in the *M. bovis* scenario tree model:

1. Dairy:
    b) bulk tank milk testing
    c) herd test following positive bulk tank milk test
    d) passive surveillance
2. Non-dairy:
    a) herd test of a random proportion of herds
    b) abattoir testing of a random proportion of herds
    c) passive surveillance

Dairy herds are divided into small, medium, and large.

The TAG is aware that the non-dairy sector classification, while driven by limitations in the farm ID systems used in New Zealand, actually covers a quite heterogeneous group of cattle producers that includes beef cow-calf, dry dairy cow grazing, dairy beef production, heifer rearing, calf aggregators, and lifestyle block herds. The likelihood of infection in these different groups varies widely based on detections to date. The scenario tree model does take herd type into account to a limited extent (non-dairy herd types: beef breeder, commercial, lifestyle). However, consideration needs to be given as to whether this approach adequately represents the New Zealand situation and is appropriate for planning future surveillance.

As it had not been implemented at the time the report was done, beef sector surveillance is not adequately described or included in the STM e.g., neither surveillance of animals entering the beef feedlot nor sampling based on the TB programme (non-random) are explicitly included.

The relative risks of infection across the various nodes containing cattle herds were based on either data or an expert opinion approach or a combination of both. In the interests of transparency, it would be useful if the source of parameter estimates were clearly documented (e.g., in Appendix 1 of the report).

It is now more than 12 months since this model was parameterised and since then more data have become available and understanding of risk pathways has improved. The TAG recommends that MPI epidemiologists and industry experts revisit the assumptions and parameter values used in the model. Specifically, the estimates of sensitivity and specificity of BTM testing seem too low, it is unclear whether the test characteristics for the BTM apply to a single point in time milk sample or to the monthly sampling considered collectively across a year. The within herd design prevalence of 5% for non-dairy herds appears high as discussed above. Given this, reassessment of the model, inclusion of other surveillance streams (i.e., beef feedlot surveillance, dairy heifer surveillance, beef sires used in the dairy industry), and alternative weightings of the surveillance streams may be found to be cost effective relative to the recommendations from the Ausvet report.

When using estimates of diagnostic sensitivity and specificity of the various diagnostic tests in STM or other modelling, careful thought is required as to the underlying disease condition that the test performance is being assessed for. For

estimates of sensitivity and specificity derived by applying the test to 'gold standard' subjects, this is usually clear. However, when sensitivity and specificity are estimated using latent class models, the latent disease condition is implied mathematically based jointly on the prior distributions and data. The biological attributes of this implied condition must be inferred based on consideration of relevant biological aspects of the condition and the diagnostic test(s). For PCRs, the latent disease condition is usually clear (e.g., current presence of DNA or RNA fragments from the target organism) but for serological tests where a) infection does not persist for life, b) antibodies develop only sometime after onset of infection, c) antibodies persist after the subject is longer infected, and d) antibodies do not persist for life, the latent disease condition is less evident. It is clearly not detecting the current presence of the target organism but rather is past exposure to the target organism within a variable time period (varying between subjects). The latent disease condition in turn affects choice of design prevalences in modelling because the design prevalence must be the prevalence of the latent condition that the tests are aiming to detect. For *M. bovis*, as the latent conditions vary between PCR and serological tests, the latent condition may be clearer for latent class models that use only one of these two types of tests. The TAG understands that previous work assessing durations of detectable levels of antibodies after exposure is being reviewed and recommends that design prevalences for modelling be defined based on understanding of the latent condition informed by this review. There are further complexities in inferring the latent condition from latent class modelling of the BTM ELISA (variation in prevalence of the animal level latent class, variation in milk yields between cows, presence of infection in heifers or dry cows but not in lactating cows in a herd, lactating cows not contributing milk to the bulk tank etc). Sensitivity estimates of the BTM ELISA obtained from variously defined gold standard infected dairy herds (using only infected herds first detected from network surveillance) should be considered along with any estimates from latent class modelling.

8 Supplementary task

Subject to available time and resources, review the paper '*Reducing the risk of Mycoplasma bovis (M. bovis) transmission by breeding bulls*' and provide technical guidance on the appropriateness of the measures proposed in this paper.

- This paper was commissioned by the Chief Science Advisor from an external contractor, and the programme would value independent advice from the TAG on the extent to which breeding bulls pose a challenge to establishing absence of the *M. bovis*.

- Examine and consider the proposal for active surveillance of breeding bulls and provide technical advice on whether this is likely to be an effective use of resources. If required, the TAG may interview the author, as well as epidemiologists and farming system experts from in the programme, DairyNZ and Beef+Lamb New Zealand.

Time constraints preclude detailed assessment of this document. The TAG agrees that breeding bulls are a potential source of transmission into dairy herds. However, the TAG understands that there is only one documented case of infection of a dairy herd by introduction of bulls. Hence it appears that the risk of this pathway is relatively low. The TAG recommends that a risk assessment of this pathway be undertaken, surveillance of bulls introduced to dairy farms be modelled in the scenario tree model, and the cost effectiveness of such a surveillance stream, above and beyond the BTM surveillance stream, be assessed on a biological and a cost/benefit basis.

9 References

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10 Appendix 1: Key issues for proof of absence

10.1 The road to freedom

Critical factors that have been identified in successful disease eradication programmes include agreed final outcome, industry commitment and financial support, a business model for programme planning, implementation and review, consistent and transparent technical standards, and objective measures of programme progress (Moore et al. 2015). Detailed forward planning should include long-term goals, interim targets, likely activities, and associated budgets.

When a disease is subject to an eradication programme, it is usual to consider the process in terms of phases. A simple example of this process may include three phases (Table 1). Typically, the 'control' phase is aimed at containing and eliminating the infection. Once the disease is thought to be eliminated (or at least reduced to a very low level), a period during which surveillance is used to collect data to support the case for disease freedom follows. This is commonly referred to as the 'provisional freedom from infection' phase. Once sufficient evidence has been accumulated, disease absence can be declared. This is a relatively simple illustration of the process and, depending on the nature of the disease, there could be additional steps in the process.

Table 1. Example of a phased approach to achieving disease freedom

Phase	**Surveillance activities**	**Control activities**
1. Control	Delimiting extent of infection Identify infected herds Casing and Tracing Understand disease spread Identify risks	Deal with infected herds Manage transmission risks
2. Provisional freedom	Monitoring the population through general and targeted surveillance Collecting data to support case for disease freedom Identify any residual infections	Deal with any residual infections Manage risk of reintroduction Collate surveillance data
3. Absence	Cease disease-specific surveillance programmes	The disease is treated as an exotic disease

While understandably work to date in the *M. bovis* programme has focussed on containing and removing the infection during the control phase, it is important that appropriate planning for transitioning to the subsequent phases of the response is

undertaken. The TAG recommends that the programme undertake this forward planning to ensure that it is well prepared, and stakeholders have a clear understanding of the way forward. This is because there will be significant operational, administration, logistical and communication issues that will need to be managed as the response moves forward. Of particular importance is managing stakeholder expectations about the timing, milestones and what may happen in the different response phases. For example, while reaching the end of the control ("delimiting") phase is an important milestone, it does not mean that the infection has been eradicated *per se*. Rather it means that all known infections have been dealt with and there is a high level of confidence that the disease risk has been reduced to a very low level. Given the epidemiology of *M. bovis*, inherent limitations in the ability to trace all animal movements, imperfect sensitivity of diagnostic tests and limitations of sampling approaches, it is necessary to undertake a further period of surveillance to collect the data necessary to support the case for freedom. Note that during this 'provisional freedom from infection' phase, it is possible that residual cases of infection may be found. It is important to be aware of and plan for these events. Finding new infections in this phase does not mean the programme has been unsuccessful, rather it should be recognised that these are expected, the programme is on track, and the surveillance measures put in place are doing their intended job.

The TAG acknowledges the approach outlined in the document entitled 'The definition of eradication' prepared by Mary van Andel (on 30 October 2019) which provides information on the need for a staged process to declare absence of *M. bovis* and indicates the end of the eradication programme, discusses of possible criteria for the transition between stages, and outlines the use of STM to optimise the surveillance programme that will provide confidence in freedom from infection. This document lays the groundwork for a staged process. Key points from this document include:

- Use of the term 'absence of disease'
- Confirmation that there is no OIE process for declaration of freedom from *M. bovis* as *M. bovis* is not an OIE-listed disease
- Outlines a surveillance-based process to provide confidence that *M. bovis* is absent from New Zealand by using scenario tree modelling.
- A project to estimate the current sensitivity of the whole surveillance programme and up-skill the programme epidemiologists to use scenario tree modelling to update sensitivity of the surveillance programme in the future.
- States that delimiting must be complete before moving to a "provisional absence" phase.
- Discussion of the criteria to be met prior to declaration for provisional absence should include consideration of:
    - The appropriate time period for this phase as dictated by the farming calendar. o It should be at least 1 calving for both autumn and spring calving populations.

- 18-24 months after the completion of the delimiting phase seems reasonable, as the replacement dairy heifers have commenced lactation and hence potentially detectable through BTM testing.

Following the establishment of provisional absence, there is an additional 3-5 years of national surveillance to provide sufficient negative surveillance to establish absence.

The TAG broadly supports the approach outlined above but recommends that further detail and planning be undertaken. Additionally, due to the potential interval between calves being infected around birth, and not lactating for 2 years, the interval between the final known cases and declaration of "provisional freedom from infection" needs to be 2 years after the last case is reported if the programme is reliant on bulk tank milk testing to detect infected replacement heifers. A long period of surveillance during the provisional absence phase will be required due to diagnostic limitations. Similar long periods of surveillance have been required in other animal disease eradication programmes such as bovine tuberculosis, brucellosis, hydatids, etc. The TAG suggests that 'freedom from infection' is a better term than 'absence of disease' given the low incidence of clinical disease with *M. bovis* and hence the risk that it is perceived by some that lack of clinical disease is equivalent to freedom from infection.

10.2 When should the programme transition from the control (delimitation) phase?

This is a key milestone in the journey to eradicate *M. bovis* from New Zealand. The TAG considers that it is important that planning this transition is well thought through and planned for. The TAG believes that this transition should not be specified in terms of a fixed time or expected date, instead it should be based on a technical assessment by animal health authorities as to when they are confident that the disease has been successfully controlled. As such, key indicators which should be considered include:

- No known infected herds in New Zealand. (NB freedom from infection cannot be declared if infected animals/herds are known to be present)
- All scheduled casing and tracing have been completed
- All identified high risk premises have been assessed
- There have been no new cases identified after 'X' period, during which 'Y' rounds of BTM testing and beef surveillance have been completed. Animal health authorities should specify 'X' taking into account the epidemiology of *M. bovis* under New Zealand conditions and the known performance of the surveillance system. TAG suggests that historical distribution of the observed time from infection to detection is an important metric to consider. Available data suggests there is potentially a lag phase from

when a herd is first infected until it is detected which may be two years or longer.

When the programme transitions to the provisionally-absent phase, the focus of surveillance shifts from finding cases to monitoring the population and assembling data to support the case for freedom from infection. Some changes to components of the surveillance system may need to be made with a focus to more risk-based surveillance . The TAG supports the use of scenario tree modelling to assist with this planning and this is discussed in more detail under TOR 3. Anticipating these changes will assist future planning and resource allocation.

Given the known limitations in diagnostic test performance and sampling regimes, New Zealand production practices as well as issues with the ability to identify and trace all livestock movement (NAIT data), it cannot be assumed that all *M. bovis* cases have been found in the control phase. It is possible, even probable, that there are unrecognised infected herds still present in the population. As such, procedures will need to be in place during the 'provisionally-absent' phase to ensure a rapid response in the event that new detections are made.

Careful consideration needs to be given as to how to manage any new detections in the provisional freedom phase – small numbers of isolated cases can be treated as 'incidents' which are not unexpected and are managed as part of 'business as usual'. However, should appreciable numbers of cases be detected, particularly if there is evidence of active spread over wider areas, then the premise that the disease has been controlled may come into question.

10.3 Transitioning from provisional freedom to freedom

After a period of monitoring with no new detections in the face of the surveillance, New Zealand declares freedom. At this point the specific *M. bovis* surveillance programmes cease. Effectively *M. bovis* is then treated as any other exotic disease.

When does New Zealand declare freedom from *M. bovis*? Technically it is not possible to prove freedom, rather it is necessary to generate adequate evidence to provide assurance that the infection is no longer present. Ongoing negative surveillance builds confidence. Time is a key ally here as the longer you go without seeing any cases the more confidence you can have. The TAG recommends that the length of the 'provisionally-free' phase and transition to *M. bovis* freedom phase should be based on sound epidemiological principles. Scenario tree models can be used to estimate the 'probability of freedom' taking into account data collected through surveillance streams that have been in place to provide a quantitative assessment on which to base this decision.

10.4 The challenge of demonstrating disease freedom

Freedom from infection is identified as ‘the absence of a pathogenic agent in the country, zone or compartment’ (OIE, 2019). The challenge of demonstrating disease freedom is to provide evidence that the disease is absent under conditions where, if it is present, it may be at a very low level and therefore very difficult to detect. Due to imperfect measurement methods and the impracticality of testing every animal in the population, it is not possible to prove absence of disease with absolute certainty. While it is not possible to “prove” freedom, it is possible to describe the level of confidence that we have, based on repeated observations of many non-infected animals. The use of terminology such as ‘testing to demonstrate freedom from infection’ is misleading and it has been suggested that it should be replaced with terms like ‘testing to substantiate freedom from infection’ (Paton et al. 2006).

When dealing with freedom from infection, the question to be asked is: *Is the country free from infection?* Or more accurately *How confident are we that a country is free?*

Demonstrating freedom from infection involves providing sufficient evidence to show that if a particular pathogen is present, it is present in less than a specified proportion of the population (design prevalence) at a given level of statistical confidence. This can be done by testing for the presence of infection in a herd by sampling a suitable number of animals, and if they are all negative, we can conclude with a certain level of confidence that the herd is not infected at the design prevalence (Schuppers et al. 2012). Most approaches address this issue by estimating sensitivity of the surveillance system. Surveillance sensitivity is a conditional probability – the probability that the surveillance system would find the disease, given that the country is infected at the specified design prevalence. This seems somewhat counterintuitive - we attempt to show that a country is free by assuming that it is infected. For most people, probability of freedom: (i.e., the probability that the country is free from infection given that the surveillance has not produced a positive result) is an easier concept to understand.

Note, however, that surveillance sensitivity cannot be interpreted as probability of freedom. Sensitivity is conditional on the population being infected, while probability of freedom is conditional on negative surveillance results. A different approach to estimate the probability of freedom is required, based on the sensitivity of the surveillance system and an estimate of prior probability of freedom, using Bayes’ theorem.

Applying a probabilistic approach to demonstrating freedom allows consideration of cumulative evidence (Cameron, 2012), such as taking results from different surveillance activities (structured and non-structured) and from previous (historical) surveillance into account. However, this also means that applied statistical methods, determination of risk pathways and estimation of risk ratios need to be scientifically sound.

10.5 Surveillance to demonstrate freedom from infection – an overview

Surveillance with the objective of demonstrating freedom from infection has to take into account the likelihood that the infection is still present in the population i.e., the risk of residual infection and the likelihood that the infection will be re-introduced i.e., risk of new introduction.

Historically, representative surveys have been considered the best way to gather evidence to demonstrate freedom from infection. Representative surveys are based on random sampling and have two major advantages:

- The animals tested are representative of all animals in the population (each animal in the population has a known probability of being selected and tested).
- Analysis of the survey data is relatively simple.

The disadvantages of representative surveys are that they are often both very expensive and inefficient. Efficient surveillance designs have the potential to reduce the cost of surveillance, whilst preserving the power of confirmation of freedom. Newer approaches based on non-representative sampling (risk-based surveillance) have been developed to address this issue.

Risk-based surveillance involves looking for disease where it is most likely to be present. Concentrating surveillance effort to those animals that are most likely to be infected is more efficient – by examining the high-risk groups, you have a greater chance of finding the disease (if it is present) than by examining animals at random.

Risk-based surveillance approaches are considered particularly suitable to support freedom from infection as the aim is not to provide representative estimates, but to detect disease in case the population is infected at or above the specified design prevalence (Cameron, 2012, Oidtmann et al., 2013). Risk factors are any factors that influence the distribution and/or the consequences of the infection in the population of interest. Such factors may be present at the population level, at the herd level or at the individual level, and can be used to improve the cost-effectiveness of surveillance by allocating relatively more surveillance efforts to population strata at higher risk. By looking for disease in animals or population strata that are more likely to be infected or detected, the probability of finding disease, if present, increases. However, risk-based surveillance requires prior epidemiological information and epidemiological skills in order to design and evaluate the surveillance. Equally, making inference from the results to the entire population is more difficult.

For further details see Stärk et al. 2006, Cameron 2012, Reist et al. 2012, and Oidtmann et al. 2013.

In practice, it is not uncommon for a surveillance system to utilise multiple surveillance activities to collect data about a given disease. The basic technique of calculating the combined sensitivity of complex surveillance systems made up of multiple surveillance components by calculating and combining the sensitivities of the individual components is straightforward but becomes much more complex when there is overlap between the coverage. Scenario tree models can be used to analyse complex risk-based surveillance in order to estimate its sensitivity, but this means that applied statistical methods, determination of risk-pathways and estimation of risk ratios need to be scientifically sound (see ToR 3).

11 Appendix 2: Herd-level sensitivities for non-dairy herds

When designing surveillance systems, assumptions about the sensitivity and specificity of the test used, and the expected prevalence of disease within the populations being surveilled needs to be defined. When the surveillance system is based on testing individual animals, the prevalence of infection within infected herds (the animal level "design prevalence") must be assumed. If assumptions are overly optimistic (for example the test is believed to have better sensitivity and/or worse specificity than truly is the situation, or that the prevalence of disease is assumed to be higher than it truly is within infected herds) then the risk is that too few animals within a herd, and too few herds, are actually tested with the risk of reaching the conclusion that disease is truly not present when it is, albeit at lower prevalences than expected. Three major reasons for concluding incorrectly that disease is truly not present are i) low herd level sensitivity, ii) too few herds assessed, and iii) herds assessed biased towards low risk herds.

Herd level sensitivity describes the probability that an infected herd will be detected by surveillance. Herd level sensitivity is markedly affected by prevalence of infection within infected herds. There is currently a lack of information about the prevalences of infection within infected non-dairy herds. The current Ausvet scenario tree modelling has assumed a 5% within-herd design prevalence. The TAG suggests that the prevalence may truly be lower than this in non-dairy herds, and strongly recommends that lower design prevalences be considered within the scenario tree models. The purpose of this Appendix is to assess herd level sensitivities if the within-herd design prevalence in infected herds is less than 5% and only 25-50 animals are tested per herd such as is common under the NBCSP, and to assess effects of various test sensitivities, specificities and interpretation on herd-level sensitivity at low within-herd design prevalences. Herd-level sensitivities were calculated using on-line software (*'Herd sensitivities and herd specificities for a range of sample sizes and cut-points for given herd size, Se and Sp*'; https://epitools.ausvet.com.au/herdplusthree).

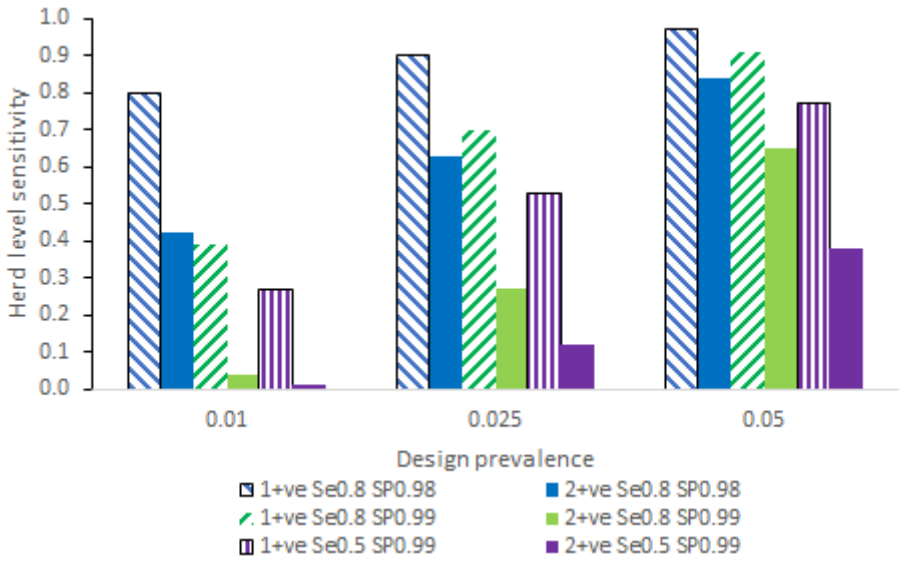
Using PCR as the gold standard has been estimated that the sensitivity of the ELISA at an SP ratio of 90 is about 79% (Mackereth and Marquetoux, 2019). However titres are likely to decline with time post exposure with those animals exposed as calves, but only then tested as one or 2-year-olds likely having a lower titre, hence the sensitivity of the ELISA may be lower. It would be reasonable to use sensitivities of between 50-80% in the models.

Early assessments suggest that the specificity of the ELISA at animal level may be in the order of 98%. However, using the data provided in the document provided to the TAG entitled 'Overview of the National Beef Cattle Surveillance programme 8 Nov 2020', from a total of 80,557 blood samples, 52 were ELISA positive and hence the apparent specificity is at least 99.94%. This difference may be because specificity is higher in non-infected herds compared to within infected herds.

These analyses showed that, at animal-level design prevalences of 1% and 2.5%, the herd level sensitivity for detection of *M. bovis* is low if the minimum number of test positive animals required to declare the herd test positive is 2, emphasizing the importance of conducting detailed investigation of herds with small numbers tested in surveillance streams and only one test positive animal (in addition to the current approach of detailed investigation of herds with two or more test positive animals).

The results also showed that even if this is done, herd level sensitivity is markedly affected by an apparently small increase in test specificity from 98% to 99.94%, particularly at very low design prevalence. If test specificity is very high, herd level sensitivity will not be as high as when test specificity is lower. Thus, it is conservative to assume high test specificity. Finally, a decline in sensitivity of the test from 0.8 to 0.5 moderately reduces herd level sensitivity (Supplementary Figure 1). As may be expected, if only 25 of 200, or 25 of 40 animals are tested then the herd level sensitivity is lower (results not shown).

Supplementary Figure 1. Herd level sensitivity for detection of *Mycoplasma bovis* at three animal-level design prevalences where the sensitivity at animal level (Se) is either 0.8 or 0.5, and the specificity (SP) is either 0.98 or 0.9994. Additionally, herd level sensitivity is presented based on the threshold for classifying a herd as test positive of either one or 2 animals test positive. It was assumed that there are 200 animals in the population of which 50 representative animals are tested.



12 Appendix 3: Scenario tree models

12.1 Scenario tree models

In the context of supporting disease freedom, a surveillance system will frequently comprise a number of different components which provide different types of evidence that the disease is not present. The scenario tree methodology, developed by Martin et al. (2007a,b), is a way of combining multiple data sources. In contrast to the simple analysis of representative surveys, the purpose of a scenario tree model is to take into account the fact that not all animals in the population have the same probability of being infected (some are at greater risk than others); nor do they have the same probability of being detected (the sensitivity of detection is greater in some animals than in others).

A scenario tree illustrates diagrammatically the successive events from infection to detection. It describes:

- the risk that an animal or group of animals might be infected (based on the risk factors for infection and the structure of the population); and
- the way in which an infected animal may be detected, based on the structure of the surveillance system.

While relatively new, the approach is increasingly being used in the veterinary field to evaluate surveillance systems (e.g., Martin et al. 2007b, Wahlström et al. 2010, Blickenstorfer et al. 2011, Christensen et al 2011, Welby et al. 2013, Christensen et al 2016, Welby et al 2016). A scenario tree model is a tool that can be used to assist in the calculation of the sensitivity of a component of a surveillance system. Additionally, it can be used to estimate the overall probability of freedom taking into account the sensitivity of the individual surveillance system components.

The purpose of a scenario tree is to describe how different parts of the population have different probabilities of being infected and of being detected (FAO, 2014). A scenario tree model allows the user to analyse risk-based surveillance, targeted at groups that are more likely to be infected. A number of terms are commonly used when discussing scenario trees, including node, branch, outcome, limb. Each risk factor affecting the probability of infection is represented by a node and a node will have different branches illustrating all possible outcomes. Each outcome has a certain probability of occurrence. Once the nodes and branches are defined, branch probabilities must be calculated or estimated along with other model parameters. To analyse surveillance in a scenario tree model, three parameters for each branch of a risk category node are required:

- the relative risk of herds or animals in that group being infected (relative to the group with the lowest risk)
- the proportion of the population in that group

- the proportion of the surveillance system component in that group

As each individual surveillance component provides additional evidence that disease is not present if no positives are found the sensitivity of each is calculated (see FAO 2014 for details). Sensitivity is the probability that the surveillance system component will detect at least one infected animal if the population is infected at the design prevalence. The component unit sensitivity is therefore the sum of the probabilities of all the different limbs that can lead to detection. Subsequently the component sensitivities are combined to obtain an overall estimate of the performance of the entire surveillance system. Finally, the probability of freedom can be calculated using Bayes' theorem, based on the sensitivity of the surveillance system and an estimate of prior probability of freedom.

12.2 Advantages of scenario tree modelling

Scenario tree models provide a relatively easy to use tool to determine the sensitivity of the surveillance system as a whole or of individual branches (e.g., components, species, production types etc.), calculate sample size and evaluate what-if scenarios. Hence scenario tree models have utility when designing "freedom from infection" surveillance processes, but also in assessing the sensitivity of the surveillance systems during the delimitation phase.

Scenario tree models can take into account:

- Multiple data sources
- Non-random data (not just from structured random surveys)
- Probability of disease incursion-including seasonal variation
- Relative risks between different population strata
- Historic information. NB Loss of value of historical data depends on the probability of introduction and the probability of spread of undetected residual infection (Knopf et al. 2007).
- Variance and uncertainty (through the use of stochastic inputs)
- Scenario tree modelling is valuable for demonstration as stakeholders can easily follow the steps undertaken in the analysis as long as it is not too complex (Hood, et al., 2009)

12.3 Limitations

As with all mathematical models, the quality of the scenario tree model depends on the quality of the inputs and the quality of the assumptions. In the absence of quantitative data, simulated values based on expert elicitation may be a useful alternative. Outputs must then be validated through, for example, sensitivity analysis.

Another potentially limiting factor is insufficient data/evidence to inform the choice of suitable risk factors and quantification of risk ratios (Oidtmann et al., 2013).

Risk-based methods like scenario tree models make it difficult to compare surveillance systems between countries (Stärk et al., 2006) and to extrapolate the results to the general population if positive samples are detected.

Scenario tree models can quickly become very complex as more nodes are added, particularly in situations where multiple trees are constructed for different surveillance components. This may make them less intuitive and more difficult to reliably parameterise.

Scenario tree models also do not explicitly incorporate different likelihoods of transmission amongst components of the animal industries but assume homogeneous transmission.

Finally, when reporting the probability that a country is free from infection based on surveillance, the choice of the prior probability of freedom can make a very big difference. It has a big impact on the result but is difficult to choose objectively. In order to avoid disagreements about the interpretation of the analysis of surveillance, there needs to be an objective and mutually agreeable method of selecting a prior.

12.4 Alternative approaches to demonstrating disease freedom

To increase confidence that the surveillance strategy selected will meet the needs for the “provisional freedom from infection” phase of the programme, the TAG recommends that in addition to scenario tree modelling, modelling of alternative surveillance approaches using a model with a different biological basis be implemented. If an alternative modelling strategy provides similar weightings, confidence, and similar sample sizes, this would provide greater confidence around declaration of freedom from infection. A number of alternative strategies to undertaking or modelling surveillance for “freedom from infection” have historically been used (see below). Such models have been used extensively during the COVID epidemic and for modelling of bovine tuberculosis in New Zealand. Social network analysis informed risk-based surveillance could also be evaluated. This may allow assessment of whether it is more cost-effective and efficient to do network-based selection of nodes compared with other surveillance streams during the final phases of delimitation. For example, analysis could be undertaken to identify high-risk nodes e.g., high in and out degree, betweenness (links), and hence to target surveillance and interventions at these high risk nodes.

12.4.1 Active surveillance using representative surveys

Traditionally, input-based standards have been used to define the surveillance needed to demonstrate freedom. These prescribe the surveillance activities to be carried out (i.e., sampling strategy, sample size, choice of test and frequency of testing), assuming that the population properties of herds are homogeneously distributed. Representative sampling relies on a point-in-time sample of a population

to detect disease if it was present at a certain prevalence. If no disease is detected, an inference can be made that disease is absent. In conventional approaches to document disease freedom, a sample of herds is selected randomly. Randomness is necessary to ensure representativeness for inference on the population. However, simple random sampling does not take into account uneven distribution of disease risk. Random sampling can be done in strata without violating the assumption of representativeness. Multi-stage random sampling results addresses the cluster effect of herds but results in a higher total number of samples (Stärk et al., 2000). Multi-stage random sampling has become the norm when animals are kept in herds. Another major advancement has been the development of the modified binomial approximation to the hypergeometric formula as it is more suitable for small populations and allows incorporating test characteristics. However, for demonstrating freedom using surveys, a very low prevalence is required for sample size calculation and, in consequence, a large sample size is necessary and active surveillance approaches tend to be costly and resource intensive.

12.4.2 Epidemiological modelling

Epidemiological models are used to represent the logical or mathematical processes in the epidemiology of a disease and associated factors (Garner and Hamilton 2011, Willeberg et al., 2011). After specifying model input parameters obtained from real data, reviews of published literature or expert opinion, endemic or epidemic diseases can be simulated. Models may be used to analyse past epidemics or predict future transmission. Such models may be used for a wide range of purposes, such as to inform national, technical, and administrative needs, address scientific questions, provide epidemiological understanding, and support international, political, and trade-related decisions (Willeberg et al., 2011). Models can vary from simple deterministic mathematical models through to complex spatially-explicit stochastic simulations and decision support systems (Garner and Hamilton 2011).

Simulation models provide maximum flexibility. For example, whilst scenario tree models assumes two infection states (infected, not infected), simulation models allows accounting for different infection states (e.g., susceptible, non-susceptible, latent-infected, lowly infectious, highly infectious, and clinical disease), various infection routes and different design prevalences. In terms of supporting disease freedom, models may be used to calculate the surveillance system sensitivity or probability of freedom, assess the cost of surveillance, calculate sample sizes, estimate epidemiological parameters), estimate the time needed to confirm disease freedom and simulate the spread of disease.

While epidemiological models provide a lot of flexibility and ability to study a wide range of scenarios, their complexity may make it difficult for stakeholders to assess the adequacy and validity of the model structure and input parameters.

12.4.3 Bayesian belief networks

Scenario tree models were criticized by Hood et al. (2009) because of their potential complexity. This author described how a Bayesian belief network (BBN), a method already used for modelling ecological systems, could be applied to deal with complex surveillance systems in a better way. Bayesian belief networks are a convenient mathematical way of representing probabilistic and often causal dependencies between multiple events or random processes. A Bayesian belief network is a computational model that is based on graph probability theory. The structure of a Bayesian belief network is represented by a Directed Acyclic graph. Compared to scenario tree models, Bayesian belief network provide a compact diagram of the structure used, simplify calculations, and extend the range of software that can be used.