Assessment of *Mycoplasma bovis* response in New Zealand July 2017 to April 2018

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Technical Advisory Group

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Key Findings and Recommendations

1. There are almost certainly some currently infected but undetected dairy herds, but the precise number cannot be specified.
2. The number of beef herds that are currently infected is probably low but there is some uncertainty about this.
3. It is unlikely that there are currently hundreds of infected but undetected dairy herds.
4. It is also unlikely that there are large undetected clusters of infected dairy herds (groups of herds infected directly or indirectly from the same source herd).
5. Eradication currently appears feasible based on understanding of the current distribution of *M. bovis* in cattle in New Zealand.
6. However, there may be other reasons why eradication is currently not feasible (including resource availability and understanding of the social and economic implications of such a programme etc etc). We have not considered these other factors in our deliberations.
7. Any eradication program will need to run for at least five years and expectations should be managed such that stakeholders are not surprised when previously undetected infected herds are identified for a number of years. These will be both currently infected but as yet undetected herds, and as yet uninfected herds that have become infected.
8. Because of uncertainty about the current distribution of *M. bovis*, if eradication is to be attempted, the TAG recommend a staged process with a go/no go decision point in late spring 2018. With adequate surveillance and robust animal movement data between now and then, a lot more will be known about the distribution of *M. bovis* at that point.
9. Robust modelling of the distribution of *M. bovis* under various control strategies should be undertaken as soon as possible to inform eradication and surveillance planning. All modelling should be subject to independent peer review, and given the complexity of the context (i.e. importance of livestock movements on the extent and distribution of the outbreak) various modelling approaches considered before major decisions are made. Additionally, economic modelling should be undertaken to allow estimation of the plausible ranges of economic impacts of the disease under various control strategies.
10. Surveillance early in lactation 2018 is critical to understand how many more infected places (IPs) there are.

11. The definition of success of any eradication or control programme should be clearly and explicitly stated in quantitative terms and agreed to by all stakeholders before the programme commences, as this will determine the design of, and commitment to, the programme.
12. A full eradication and surveillance plan should be developed and costed. The approach will likely differ between the dairy and beef industries.
13. Given movements of animals between the beef and dairy industries, any eradication programme should include both industries.
14. Clear lines of reporting and responsibility need to be established as part of any eradication/surveillance plan.
15. There is a clear need to improve animal recording nationally by improving implementation, monitoring and compliance with NAIT. Additionally messaging around biosecurity, disease identification, recording and reporting, and improved professional oversight of disease incidence on farm are required.
16. The human and animal health and welfare costs are not to be underestimated and strong consideration must be given to these factors in any decision-making.
17. Further work is required to optimise cut-off points for tests and to design appropriate surveillance systems. Techniques that allow estimation of sensitivity and specificity in the absence of a gold standard should be used to provide better estimates of test characteristics for such programmes.
18. The test characteristics of the milk ELISA should be defined at herd level as a matter of urgency.
19. Additionally, novel tests with higher sensitivity and specificity (i.e. the MilA antibody ELISA) are likely to become commercially available in the near future and these should be assessed and implemented if appropriate, as soon as possible.
20. While repeated testing of a herd may increase sensitivity, the amount of additional information provided by repeated testing of the same herd should be quantified to optimise resource use.
21. If a national program is to be implemented, a regional or national census of dairy herds using bulk milk samples (both bulk tank and sick cow milk) tested by both PCR and ELISA during early lactation should be implemented for each of the next five years.
22. The sensitivity of ELISA testing of animals exposed many months before testing (for example following exposure to infectious milk or in calf rearing sheds) needs to be defined.
23. A strategy for dairy farmers to manage importation of service bulls is required. It is likely that testing of individual bulls, even repeatedly, is likely to be of low sensitivity. Thus options include use of AI only, or some form of bull farm assurance of disease status.
24. Forward trace farms that have not been test positive thus far should have a higher level of scrutiny. Repeated bulk milk testing of these farms over the next 3 to 5 years should be undertaken in accordance with the surveillance plan developed.
25. Testing capability of all laboratories nationally needs to be improved and clear guidelines provided as to the laboratory's responsibilities where test positive bulk milks, quarter level milk samples or other tissues are detected.
26. The proposed cleansing, disinfection and stand-down protocols appear thorough and consistent with international best practice.
27. Reducing the disinfection protocol to include a single disinfection step is unlikely to significantly increase the risk of re-infection of newly introduced cattle after repopulation, although when decontaminated farms are repopulated with cattle, these cattle should be frequently tested, so that it becomes quickly apparent if the decontamination was ineffective.
28. It is unlikely that the environment provides a major source of infection.
29. Data on survival of *M. bovis* in effluent ponds and following spreading of effluent onto pasture is lacking, so firm recommendations about effluent management can't be made at this stage.

Introduction and commentary

The Technical Advisory Group (TAG) commends the efforts of MPI and others for the huge amount of work that has gone into this response thus far. It is clear that there has been an enormous effort on behalf of field staff, laboratory staff, and managers to deal with a very complex and evolving situation.

The TAG were given some specific questions which will be addressed in detail later in this document.

It is important to first provide some broader context and a holistic view of the TAG's current understanding of the disease as this underpins our recommendations.

The TAG generally believes that the surveillance data and molecular epidemiology support the notion that *Mycoplasma bovis* has been recently introduced and is probably not present in a large number of herds. However, there is variation in the degree of certainty amongst TAG members as to how many as yet undetected infected places (IPs) there may be. Of concern are the likely number of unrecorded young stock movements that may have occurred. It is very likely that there are additional IPs that are not yet identified. Associated with this are concerns about the limitation of the current testing methodologies namely the low sensitivity of PCR to detect organisms in the bulk tank milk and the undetermined sensitivity of serology in groups of non-lactating animals, particularly a year or more post-exposure, as they may have low titres and hence be undetectable.

The TAG believes that eradication remains feasible given the current understanding of the incursion. However, there is a degree of caution about the probability of success of eradication given the escalating requirement for resources to deal with the growing number of trace forwards, as well as dealing with a potentially substantial number of test positive herds in spring 2018, and uncertainty about unknown animal movements and unknown infected herds.

To this end, the TAG strongly recommends that robust economic modelling be undertaken once eradication and surveillance planning has occurred . A clear stop/go point must be set once costing of eradication and surveillance are clearer. The TAG would like to see the economic analyses that have been done (it is our understanding that several more analyses have been done since the BERL report from late 2017). While we understand that our advice is technical (and this recommendation may appear to be outside the scope of this group), some understanding of the economic context is critical for an informed response from the TAG.

It is the opinion of the TAG that there will be more herds identified as test positive in spring 2018. This is because it is likely that some currently unknown groups of rising two-year-olds that are infected will calve into naïve herds. Given limitations of surveillance based on clinical signs and of serology in animals exposed quite some time prior to sampling, it is believed likely that such carrier animals will have recrudescence of disease, resulting in potential shedding of *Mycoplasma bovis*, transmission within naïve herds, and hence those herds becoming PCR positive in bulk milk and/or positive on ELISA. Surveillance early in lactation 2018 is critical to understand how many more infected places (IPs) there are. The same circumstance will exist in Spring 2019 as 2017 born calves enter herds, but first-class animal movement recording will mitigate much of the impact of this.

There remain a number of technical issues around testing, including the sensitivity and specificity of the milk ELISA both at animal and herd level, as well as serum ELISA sensitivity at animal and herd level and sensitivity of culture and/or PCR at post-mortem. Further work is required to optimise cut-off points for tests and to design appropriate surveillance systems (number of animals, timing etc.) based on a better understanding of test characteristics. It is likely that improvements in test performance can be achieved by optimising cut-off points, timing of sampling, and use of tests in series or parallel to

optimise outcomes. Use of techniques such as latent class analysis, which allows estimation of sensitivity and specificity in the absence of a gold standard, should be used to provide better estimates of test sensitivity and specificity. A number of groups in New Zealand and Australia have the ability to do this type of analysis and these groups could be engaged to do this work if needed. Additionally novel tests with higher sensitivity and specificity (i.e. the MilA antibody ELISA) are likely to become available in the near future and these should be assessed and implemented if appropriate, as soon as possible.

The TAG believe that, even where strict movement control and eradication is implemented, new infected places are very likely to be identified for a number of years. This is related to the above-mentioned latency of infection and unrecorded movements. Any eradication program will need to run for at least five years and expectations should be managed such that stakeholders are not surprised that previously undetected herds are identified for a number of years. While this disease is not a zoonosis or a notifiable disease under OIE, clarity around how success of any eradication program is defined is a priority. An approach might be to define the degree of certainty about absence of disease nationally that would be acceptable to key stakeholders. For example, the objective might be 95% confidence that the national herd prevalence is less than 2%. Given such parameters, long-term surveillance programs with sufficient number of herds, animals and test types can be designed and funded ahead of time to ensure delivery of such a surveillance system.

Given the current uncertainty and the likelihood of further IPs being identified, the TAG believe it is prudent to review any eradication decision once data from spring 2018 is available. Were a large number of previously undetected IPs to be identified, eradication may become untenable, and alternative approaches (such as an industry control programme) should then be considered.

The route of introduction remains unclear and hence there remains a high probability of reintroduction if the route is not identified and the risks mitigated.

Modelling is a useful tool to identify the potential trajectory of the epidemic, as well as identifying knowledge gaps. The greatest value of such modelling currently lies in its predictive value and the ability to model different intervention scenarios. Models are an imperfect representation of reality and a number of different modelling strategies exist. Any modelling should be subject to peer review, and different modelling approaches must be considered before major decisions are made. Additionally economic modelling should be incorporated. It remains unclear to the TAG at this time point what plans there are for modelling, and how and where those results are to be used. The TAG would appreciate information on this and a briefing as to progress on model development. The TAG believes the modelling can make important contributions to decision-making and in addressing some of the questions that the TAG has been asked to address. Thus the TAG believes modelling outputs could be useful and should be available for review by the group. Modeling depends on making inferences on causative relationships. There is concern that if inferences from the model are substantially different from those of the TAG then uncertainty amongst stakeholders will result. This can increase the risk that decisions are either not made because of this uncertainty, or the wrong decisions are made. Thus alignment between communications from the modelling process should be considered in conjunction with the TAG responses.

Independent of the short term decisions that are made by MPI and others, there is a clear need to improve animal recording nationally by improving implementation, monitoring and compliance with NAIT. Additionally messaging around biosecurity, disease identification, recording and reporting, and improved professional oversight of disease incidence on farm are required.

The TAG are aware of the potential animal and human welfare implications of ongoing control and eradication. The human and animal costs are not to be underestimated and strong consideration must be given to these factors in any decision-making.

Question 1) Given the information MPI currently holds, how confident can we be that we understand the true distribution of this disease in New Zealand?

Interpretation of the question

We have taken this question to be asking whether we understand the current distribution of the organism, *M. bovis*, in cattle in New Zealand.

'current': While we focus on current distribution, we do also make some comments about possible future distribution.

'distribution': By distribution, we have focused on numbers of undetected infected herds. Undetected infected herds could be herds infected from any previous undetected *M. bovis* incursions into New Zealand, and undetected herds infected directly or indirectly from the recent incursion, whether part of a 'cluster' (a group of herds infected directly or indirectly from the same source herd eg s and the 9 IPs that trace from it, s and the 13 IPs that trace from it, and s and the 3 herds related to it) or not. We have not focused on the spatial distribution of such herds other than to make the comment here that any currently undetected infected herds are most likely to be in regions where infection has already been detected, given that cattle movements are more likely to be to closer herds than to more remote herds. It is, of course, not possible to predict the specific locations of any currently undetected infected herds.

'in cattle': Although *M. bovis* has been isolated from other species, it is considered highly host-adapted, and that cattle are by far the predominant reservoir of *M. bovis*. While *M. bovis* can 'spill over' into other species, we are not aware of evidence of *M. bovis* from other species infecting cattle. While the organism can survive in the environment for some time, it is generally considered that the major *M. bovis* reservoir is infected cattle. We note that the current experience of the disease in New Zealand is mostly associated with dairy herds, but infection may be present in beef herds and identifying infected beef herds will require alternative diagnostic approaches.

We have used information MPI has provided along with other knowledge to address this question.

Conclusions from the Technical Advisory Group

1. There are almost certainly some currently infected but undetected dairy herds.

2. The precise number of such herds can not be specified.

3. However it is unlikely that there are currently hundreds of such herds, nor large undetected clusters of infected dairy herds (groups of herds infected directly or indirectly from the same source herd).

4. The number of beef herds that are currently infected is probably low but there is some uncertainty about this. Given movements of animals between the beef and dairy industries, any eradication campaign must consider surveillance of beef animals.

5. It is likely that, even under an eradication scheme, there will be ongoing newly identified infected herds for some years. These will be both currently infected but as yet undetected herds being identified, and as yet uninfected herds becoming infected.

Rationale for these conclusions

Clinical disease patterns

Limitations of this type of evidence

While, infection has been associated with clinical disease in some herds, there appears to be minimal or no recognisable clinical disease attributable to M. bovis in most infected herds. Thus, the absence of overt clinical disease attributable to *M. bovis* in a herd provides minimal evidence that the herd is free of infection.

Further, caution is required when using clinical disease patterns as evidence, as there is underreporting of clinical disease by herdowners. For such a herd to come to the attention of a veterinarian, managers of infected herds would need to recognise abnormal disease incidence or presentation and seek veterinary advice. It is considered likely that such a report to a veterinarian would prompt further investigation as awareness of *M. bovis* as a potential cause of disease is high.

Evidence from clinical disease reports that the prevalence of *M. bovis* is not high

If the national herd prevalence in dairy herds was high (i.e. if a high proportion of New Zealand dairy herds were currently infected), some of those infected herds (albeit probably only a small proportion) would be expected to have exhibited clinical disease patterns suggestive of *M. bovis*, and some of these would have come to the attention of veterinarians. Other than in some known infected herds, dairy cattle veterinarians have not commonly reported such clinical patterns. Clinical veterinarians are now well aware of the clinical syndrome and are not generally reporting it.

For example, if 2 in 33 newly infected milking herds exhibits substantially abnormal clinical disease patterns (as seems to be the case with current known infected herds) and 75% of those epidemics are reported to a veterinarian, if a further 100 herds (in addition to the 33 known infected herds) had become infected, we would expect 5 such epidemics (100*(2/33)*0.75) to have come to the attention of a veterinarian. Given the dramatic clinical disease patterns, it is considered likely that veterinarians would have communicated about these epidemics.

In addition, there was a syndromic surveillance project undertaken s 9(2)(a) whereby clinical veterinarians across New Zealand were asked to identify herds with

increased incidence of lameness, mastitis, bulk milk somatic cell count or calf disease, and these herds were tested. No additional herds were identified this way. In total, 89 herds were identified and subjected to testing for *M. bovis*.

Tracing

Evidence that there are at least some currently infected but undetected dairy herds

In addition to currently identified herds (most of which were identified from trace forwards), there is likely to have been a high number of unrecorded (and hence untraceable) calf movements from infected herds (and particularly from s 9(2) which is proposed as the first herd to be infected). Thus, it is likely that currently there are herds that were infected indirectly from the recent incursion but are as yet undetected.

There are no documented animal movements onto s 9(2)(b)(ii) that account for their infection status. It is possible that they did, in fact, receive cattle from other known infected herds but the relevant trace information was not available. The fact that trace information is not always complete is consistent with our conclusion that there are currently infected but undetected farms. A further possibility is that s 9(2) were infected as part of a previous incursion into New Zealand. However genetic analyses and surveillance results do not support this (see below).

The likelihood of a wide distribution of infection within the country is increased by the fact that for *M. bovis* worldwide and in New Zealand , infected herds tend to be those with more movements of animals to and from other herds.

The inability to identify source movements on known IP's confirms the uncertainty that exists around the movement of livestock more generally.

Evidence that it is unlikely that there are currently hundreds of infected but undetected dairy herds

As most of the currently identified infected farms were detected based on tracing animal movements rather than surveillance (31 of 33), this supports the view that animal movements (and movement of milk for feeding to calves) have been the major methods of transmission between herds. If there were other common unidentified pathways of transmission of *M. bovis* between herds, our confidence about the distribution of *M. bovis* would be markedly reduced.

Genetic analyses of *M. bovis* isolates

Evidence that it is unlikely that there are currently hundreds of infected but undetected dairy herds

There is good confidence that the introduction causing the currently detected infections is relatively recent. The whole genome sequencing work strongly supports a recent (i.e. no more than 2 to 3 years) introduction of a single genetic strain, as evidenced by the fact that all currently sequenced isolates (n=37) are relatively closely related.

Evidence that there are at least some currently infected but undetected dairy herds

Large numbers of cows move amongst farms around June 1 each year associated with changing sharemilking contracts ("gypsy day"). There have been 1 or 2 Gypsy days since the estimated incursion date, which increases the likelihood that there are undetected infected herds.

Evidence that it is unlikely that there are currently hundreds of infected but undetected dairy herds

The National Milk Surveillance Programme PCR tested milk to identify dairy herds that had at least one cow with an infected quarter lactating at the time of the testing. This milk-testing programme consisted of testing three milk samples, with a sample collected every 10-14 days: one bulk milk sample as supplied to processors and two samples of milk withheld from supply as it contained milk from cows under treatment for mastitis or other disease. The results from this programme were used to estimate the total number of infected herds. We note that the test characteristics of these tests at the herd level are not well defined, so estimates of sensitivity have been used. If the joint herd level sensitivity of this regimen was only 0.1, assuming near perfect herd level specificity, if the herd prevalence was 0.004 or greater (ie at least 47 herds of the 11,650 dairy herds in New Zealand), at least 6 herds would be expected to have had a positive result in one round of testing (i.e. if all herds were tested under this regimen once). For example, if the herd prevalence was 0.01 (ie at least 117 infected herds in New Zealand), at least 13 herds would be expected to have had a positive result (see table below). All New Zealand herds were tested in the first round of testing and only 1 herd had a positive result and a further 3 had PCR suspicious results.

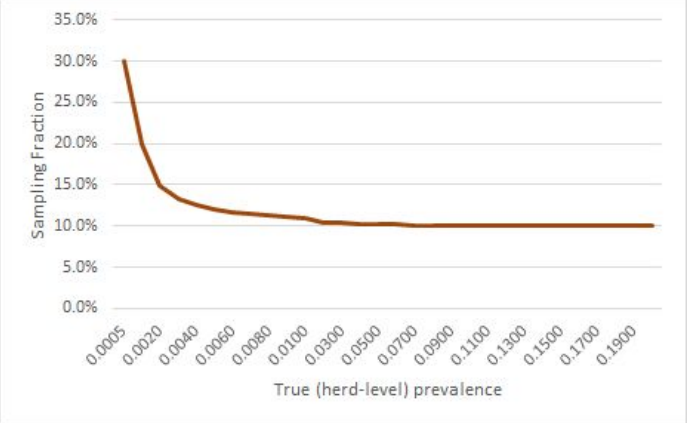
True herd prevalence [1]	No. infected dairy herds in NZ[3]	Joint sensitivity of bulk milk and sick cow milk PCR regimen [3]	Joint specificity of bulk milk and sick cow milk PCR regimen	Expected number of dairy herds positive [3]
0.00050	5.8	0.1	0.9999	1.7
0.00100	11.7	0.1	0.9999	2.3
0.00200	23.3	0.1	0.9999	3.5
0.00300	35.0	0.1	0.9999	4.7
0.00400	46.6	0.1	0.9999	5.8
0.00500	58.3	0.1	0.9999	7.0
0.00600	69.9	0.1	0.9999	8.1
0.00700	81.6	0.1	0.9999	9.3
0.00800	93.2	0.1	0.9999	10.5
0.00900	104.9	0.1	0.9999	11.6
0.01000	116.5	0.1	0.9999	12.8

[1] Proportion of dairy herds with at least one cow with an infected quarter lactating at the time of testing.

[2] Excluding those known infected before Milk Surveillance Programme commenced

[3] Test positive is defined as where any one of the 3 milk PCR tests undertaken 10-14 days apart for each herd were positive. Calculated assuming expected number of positive herds = Apparent Prevalence × 11,650 herds, where Apparent Prevalence = True Prevalence × Sensitivity + (1 - True Prevalence) × (1 - Specificity)

The sampling fraction (expected number of herds that test positive including false positives/actual number of herds infected) at various true herd prevalences asymptotes at 10% once the herd prevalence is >0.01 (see graph below).



These calculations describe the expected numbers of positive herds given known prevalences. As the first round of surveillance has been completed, it is also informative to instead estimate the true herd prevalence given these surveillance results. Accordingly, limited analyses were performed using Bayesian latent class models. The prior distribution of herd prevalence was set as uninformative i.e. all prevalences were assumed to have the same prior probability.

Results of these analyses are shown in the table below. If a herd is defined as positive on the basis of at least one sample PCR suspicious or positive, at a very low assumed sensitivity, the estimated herd prevalence was extremely low. The median estimate was 0.0028 equating to 33 herds (in addition to those herds known to be infected before the surveillance programme commenced). However, that estimate was imprecise; the 95th percentile was 0.0407 equating to 474 herds.

At higher (and perhaps more realistic) prior sensitivity, the estimated herd prevalence was even lower. The median estimate was 0.0006 equating to 7 herds and the 95th percentile was 0.0039 equating to 45 herds.

Further, assuming this PCR has the same diagnostic validity for all genotypes of *M. bovis*, the National Milk Surveillance Programme would be expected to detect dairy herds infected with the apparent recent incursion into New Zealand along with any herds still infected from any previous incursions.

Prior distributions (mode (95th or 5th percentile))		Posterior distributions (median (95% probability interval))		
Joint sensitivity	**Joint specificity**	**Herd prevalence [1]**	**Joint sensitivity**	**Joint specificity**
Herd positive = at least one sample PCR positive				
0.1 (0.3)	0.9999 (0.95)	0.0013 (0.0000 to 0.0191)	0.0777 (0.0077 to 0.2683)	0.9999 (0.9996 to 1.0000)
0.5 (0.8)	0.9999 (0.95)	0.0003 (0.0000 to 0.0021)	0.3978 (0.0809 to 0.7990)	0.9999 (0.9996 to 1.0000)
Herd positive = at least one sample PCR suspicious or positive				
0.1 (0.3)	0.9999 (0.95)	0.0028 (0.0001 to 0.0407)	0.0779 (0.0072 to 0.2681)	0.9998 (0.9993 to 1.0000)
0.5 (0.8)	0.9999 (0.95)	0.0006 (0.0000 to 0.0039)	0.3986 (0.0837 to 0.7988)	0.9998 (0.9993 to 1.0000)

[1] Proportion of dairy herds with at least one cow with an infected quarter lactating at the time of testing.

Evidence that the number of beef herds that are currently infected is probably low but there is some uncertainty about this

There should be discussions about whether surveillance of the beef industry, and eradication of any detections in beef herds, should be incorporated into any eradication program. There are substantial challenges in doing surveillance in beef herds but there are also possible impacts of *M. bovis* on health, production and animal welfare in beef herds, and pathways by which *M. bovis* could be transmitted from beef to dairy herds. In some other countries where *M. bovis* has been present for many years, the herd prevalence of *M. bovis* is higher in beef herds than in dairy herds. The TAG has assumed that eradication would include ongoing surveillance and eradication of any *M. bovis* detections in the beef industry. Hence, we have included these statements about *M. bovis* in beef herds.

Beef cattle surveillance has included purposive targeted sampling of cattle from properties with large numbers of movements. Approximately 1,800 cattle presented for slaughter from 720 properties nationwide were tested using conventional (rather than real time) PCR on tracheal, pharyngeal and lesion swabs and ELISA on serum. While positive and suspicious results were detected in the PCR and ELISA, all were considered false positives as these tests were not *M. bovis* specific and would have cross reacted with other Mycoplasma in New Zealand.

Properties where non lactating animals were grazed which were identified by trace forwards from known infected herds have also been assessed with serum testing for *M. bovis* antibodies, followed by PCR on tonsil swab at slaughter if depopulating non-lactating IPs.

There is a need for more extensive surveillance of animals being reared for beef and hence which will not be tested by bulk tank milk testing in dairy herds.

Many dairy herds in New Zealand use intact bulls towards the end of the seasonal breeding programme. Some of these are beef breeds. Hence surveillance of properties providing service bulls to dairy herds also need appropriate surveillance as individual bull testing is likely to have unacceptably low sensitivity.

Dairy heifer replacement stock

Evidence that there are at least some currently infected but undetected dairy herds

The National Milk Surveillance Programme would not have detected herds where young (< 2 years old) replacement stock were infected but cows were not. It is likely that there are at least some herds where this is the case.

Such herds would be detected only by testing replacement stock or waiting until these replacement stock enter the lactating herd where milk surveillance would be expected to detect those herds. The question with the latter strategy is how much transmission would occur from infected replacement stock to cattle from other herds. However, surveillance of replacement stock from herds with no particular suspicion of having *M. bovis* would demand substantial resources, and may have low group-level sensitivity. If most infected replacement stock become infected when relatively young, and there is subsequently minimal transmission within groups, it is possible that, for many infected calves and yearlings, serum titres will have waned before testing.

Biology of M. bovis

Evidence that there are at least some currently infected but undetected dairy herds

The biology of *M. bovis* infection may constrain knowledge of its distribution in New Zealand. Many cattle with infected quarters do not show clinical signs and shedding may be transient so infected cattle can evade detection by milk PCR, potentially for prolonged periods.

Evidence that it is unlikely that there are currently hundreds of infected but undetected dairy herds

Infected but PCR-negative lactating cows would result in false negative bulk tank PCR results, particularly in herds with few infected cows. However, our analyses under ‘Surveillance’ (above) incorporated relatively conservative herd level sensitivities. Also, provided infected but PCR-negative lactating cows transmit *M. bovis* to other cattle that do shed, it is likely that such herds would eventually be detected by bulk tank or sick cow milk PCR.

Further, based on New Zealand, European and Canadian experiences, most animals develop serum titres after infection. Thus, if surveillance includes ELISA testing, most herds with infected PCR-negative cows should eventually be detected.

Evidence that under an eradication programme, there will be ongoing newly identified infected herds for some years

Observations in Australia and USA suggest that clinical cases can cease for 2-3 years before reappearing, that infected herds can have low bulk tank ELISA results, and that infected cattle can remain undetected in herds for long periods. Subclinical carriage of *M. bovis* can later erupt into clinical disease in the herd. Depending on the design of any surveillance system, all of these phenomena may delay detection of infected herds, yet such herds may be transmitting to new herds in the interim.

Question 2) How does this level of confidence influence the decision making regarding the options for disease control going forward?

Conclusions from the Technical Advisory Group

1. The feasibility of eradication depends on numerous factors, only one of which is the distribution of *M. bovis* at the commencement of an eradication programme.

2. Eradication is currently feasible based on understanding of the current distribution of *M. bovis* in cattle in New Zealand. However, there may be other reasons why eradication is currently not feasible (e.g resource availability and understanding of the social and economic implications of such a programme etc.). We have not considered these other factors in our deliberations.

3. Because of uncertainty about the current distribution of *M. bovis*, if eradication is to be attempted, the TAG recommends a staged process with a go/no go decision point in late spring 2018. With adequate surveillance and robust animal movement data between now and then, greater clarity about the distribution of *M. bovis* is expected by that time point.

Rationale for these conclusions

The TAG considers dairy replacement stock (largely 2016- and 2017-born heifers) to be the population subset most likely to be infected but undetected.

The tests currently available for detection of *M. bovis* infected animals rely on expression of clinical disease, detection of the pathogen (PCR) or evidence of recent infection (antibody ELISA). The sensitivity of the PCR at the herd level may increase during times of lowered immunity in the animals, or shortly thereafter. A common cause of lowered immunity is experiencing stressful events such as calving, transport and herd mixing.

There are substantial difficulties in assessing the status of dairy replacement stock. These difficulties include the geographical distribution of the dairy replacement stock, the nature of the facilities on farm (often limited infrastructure), the low likelihood of disease expression, the low sensitivity of suitable tests, and the logistical constraints (human and laboratory) of conducting this testing now.

By late spring 2018, most 2016-born heifers will have calved, experienced the stressors associated with calving and commencing lactation, and mixed in very close contact with older cows. Thus, assuming reasonably effective nationwide surveillance of lactating dairy herds is implemented, if that cohort are infected, the likelihood of detecting infection in the lactating herds would be expected to be at least moderately high. At this time, further testing under the National Milk Surveillance Programme during early lactation will provide considerable additional confidence about the distribution of *M. bovis* infected herds. This approach, implements testing at the optimal time, and also allows time to better validate the tests that are being used (or new tests), be smarter in the use of the resources available (test reagents, personnel, etc) and provides some respite to a very stretched organisation.

By reassessing data in late spring 2018, this will provide time to improve compliance with NAIT to improve rapidity and comprehensiveness of tracing (source and spread) for any new detections.

Industry and producer support will be needed to ensure that NAIT data improves, along with implementation of appropriate monitoring and compliance enforcement measures.

Further, the number of beef herds that are currently infected is probably low but there is some uncertainty about this. If effective surveillance of beef herds can be designed and implemented by late spring 2018, this uncertainty should be markedly reduced.

Even though in late spring 2018, uncertainty will remain about the status of 2017-born dairy heifers, these additional sources of information will result in considerable additional confidence about the distribution of *M. bovis*.

Surveillance will be required for a number of years before ‘freedom of disease’ can be declared. Thus there will be some degree of uncertainty up until that time point.

The likely impacts of other factors affecting the feasibility of eradication may also be clearer by then.

Question 3) Advise on the validities of the ELISA and PCR tests for:

- Different classes of animals;
- Bulk milk in early versus late lactation?

The real-time PCR assay currently in use to detect *M. bovis* DNA has a very high specificity for all sample types. Its sensitivity on acutely infected animals (i.e. days or weeks after infection) has not been definitively assessed but it appears to be high. Its sensitivity for detection of chronically infected animals (i.e. weeks or months after infection) has not been assessed but is likely to be low given the expected short duration of detectable shedding. Bearing this in mind, it is likely to have utility in screening for herds infected in the last few months.

The use of the PCR assay for detection of infected herds by analysis of bulk tank milk samples has been assessed using data from some known infected properties. This analysis suggests a low sensitivity for detection of infected herds. However, this analysis was performed on herds in which infection has been present for an extended period of time. These herds may have had very low proportions of recently infected cows and hence low levels of shedding excretion of *M. bovis* into milk. In addition, the sensitivity was estimated on samples from herds that had already been diagnosed as infected, and herd managers may have shifted infected cows from the bulk milk supply, further reducing the sensitivity. Thus the estimate of the sensitivity of this assay for screening bulk tank milk samples probably underestimates its capacity to detect infected herds across the industry. The assay could be expected to be more sensitive early in lactation, when there is likely to be a greater number of recently infected, previously naïve animals (particularly first calf heifers) contributing to the bulk tank milk, rather than later in lactation, when infection has become more chronic and rates of excretion are likely to be lower.

In spite of the limitations of this assay, its use in surveying the entire dairy population to ensure that there has not been a significantly greater distribution of *M. bovis* than has been suspected appears valid.

The antibody detection ELISA (BioVet Bovicheck) that has been used in the survey of the beef industry and to assess the status of restricted premises has a relatively low sensitivity for detection of individual infected animals. However, the high rate of dissemination of *M. bovis* within infected herds, and subsequent high within-herd animal level prevalence, ensure that the application of the assay to confirm the status of individual herds is valid. The analysis of the data that have now been acquired during the response to the incursion has allowed considerable refinement of the interpretation of the results of the ELISA, increasing the specificity of the assay when used as a herd test without compromising its sensitivity.

Some of the analyses to estimate sensitivity of the ELISA on serum considered animals from PCR positive herds as ‘reference animals’ whereas others have presented very limited results using Bayesian latent class analysis. Interpretation of these comparisons should be made with extreme caution given virtually no agreement, above that expected by chance, between tests in a recent study (Parker et al., 2017) comparing PCR with a similar ELISA (albeit less sensitive). Such poor agreement is expected given the target analytes of these assays (antibody versus nucleic acid of the organism). Considering the data available, it is suggested that a full and well documented Bayesian latent class

analysis be undertaken, following the STARD-BLCM guidelines (Kostoulas et al., 2017), implementing a model for two conditionally dependent continuous outcome tests (using corrected OD and S/P results from two ELISAs) to estimate the diagnostic specifications and most appropriate cut-offs (Choi et al., 2006). Technical assistance is offered by the OIE Collaborating Centre for Diagnostic Test Validation Science (CSIRO AAHL, University of Melbourne and Massey University) for this task.

Little data has been generated as yet on use of the ELISA to screen bulk tank milk samples. Use of an ELISA for ongoing bulk tank milk surveys is likely to increase the sensitivity of detection of infected herds provided the ELISA can be adapted sufficiently well to this sample type, as the levels of antibody in milk are likely to remain higher for a longer period of time after infection than the levels of *M. bovis* DNA. Although the Bovicheck ELISA might also be expected to be more sensitive earlier in lactation, because there would be a greater proportion of recently infected animals in the herd, this effect will be less marked than with the PCR assay. Adaptation of the ELISA is likely to require considerable additional work by the laboratory, assessing the effect of different dilutions of the samples on the results and analysing the results of testing to identify an optimal cut-off. The ongoing bulk tank survey should provide appropriate samples for such studies to optimise the assay for this purpose, as North Island herds can be presumed to represent an uninfected population and samples from known infected herds to represent an infected population. The availability of an optimised bulk tank ELISA is likely to be of greater importance in identifying future infected herds if the decision is made to continue towards eradication.

None of the assays currently available are sufficiently sensitive at the individual animal level to allow their use for presale testing of individuals or even small groups of animals. This is likely to require an ELISA with considerably greater sensitivity for individual infected animals, such as the MilA ELISA (Wawegama et al., 2014; Wawegama et al., 2016) recently assessed by the European CoVetLab group.

Furthermore, if an eradication program is to proceed, an ELISA (or other immunoassay) with very high diagnostic sensitivity and specificity will be required in the later stages. These specifications must be evaluated in a well-documented analysis based on samples representative of those for which the test will later be applied.

Conclusions

- The data presented appear to provide reasonable estimates of the sensitivity and specificity of the bulk milk PCR and the serum ELISA.
- The sensitivity and specificity of the ELISA for milk either at a herd or individual animal level remains to be defined.
- Presently, there are insufficient data to clearly define differences in sensitivity and specificity of the tests by class of animal or stage of lactation. However, from first principles it appears logical that: the sensitivity of bulk milk PCR is likely to be higher in early lactation due to increased likelihood of shedding of the pathogen; and the sensitivity of the bulk milk ELISA is also likely to be higher from early in lactation (although 1-2 weeks later than the PCR, and persisting test positive for longer). The relationships between timing of introduction of *M bovis* to a herd, presence of *M bovis* as detected by PCR in bulk milk, and antibody titres in bulk milk remain unclear.

- Novel tests with higher sensitivity and specificity (i.e. the MilA antibody ELISA) are likely to become commercially available in the near future and these should be assessed and implemented if appropriate, as soon as possible.

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Question 4) What would you recommend as a plan for national surveillance:

- If eradication is attempted;
- If eradication is not attempted?

The preliminary documents on surveillance presented by MPI (authored by s 9(2)(a) in one case, and s 9(2)(a) in the other) require significant additional work. It is not clear if any manpower, laboratory or economic planning/forecasting has been undertaken to assess the feasibility of the proposed surveillance systems.

Additional modelling is underway by s 9(2)(a) et al using the SIR Interspread model (Stevenson et al. 2013). The preliminary report was made available to us on 9 April 2018. This report makes a number of important assumptions. These include modelling stock movement using NAIT data which is likely to grossly underestimate movements, particularly of youngstock. The model also discounts routes of transmission other than movement of animals and feeding of raw milk. The model suggests that with no government intervention, and effectively no farmer activities to limit spread, that nearly 50,000 herds will be infected over 20 years. This should be regarded as worst-case scenario as it is likely that farmers would in fact intervene to reduce risk and this predicted exponential increase in infected premises does not reflect the experience in Europe and North America. It is noteworthy the modelling suggests that more than half of the infected herds under this scenario are in the beef, not dairy industries. The rapid stamp out model results in eradication in a median of 34 months, with the median number of herds infected estimated to be 60. A less rapid form of eradication in which trace forward is discontinued and the speed of surveillance is halved, results in eradication taking a longer period of time (median = 41 months) and with a higher number of herds becoming infected (median = 76). Modelling of a strategy that is unregulated and dependent on industry-led program of enhanced biosecurity and development of a *Mycoplasma bovis* accreditation scheme with only passive surveillance fails to achieve eradication in the 20 year period in more than ¼ of the simulation runs, with a median elimination time of 113 months. It also results in a high number of herds (N = 250) being infected.

Any surveillance program will also have to include nonlactating animals. Surveillance at slaughter either completely at random, on a regional basis, or targeted at individual animals with pathology (for example joint or lung lesions) needs to be modelled.

If eradication is not attempted

If eradication is not attempted, it is assumed that management of *Mycoplasma bovis* would be devolved to some form of industry led management and accreditation process. The objectives of surveillance under such a scenario are unclear and hence cannot specifically be commented on. However, it would appear logical that some form of system by which purchasers of stock could have some degree of assurance around the *M. bovis* status may be required. If the modelling of s 9(2)(a) et al is correct, eradication would not be achieved. In this case *M. bovis* would become endemic and individual herd owners would be presumably bearing the cost of management and those purchasing stock run the risk of introduction. Under such a scenario, ongoing surveillance would not presumably be funded by government, and some form of accreditation scheme would need to be established, and

presumably funded by industry. Without regulatory control, compliance is unclear, but likely less than 100%.

Factors affecting a surveillance program

The clinical presentation of *M. bovis* infection appears to vary amongst farms, ranging from overt, high incidence, clinical disease including multi-quarter non-responsive clinical mastitis, polyarthritis, and otitis media and pneumonia in calves, through to entirely subclinical disease. Thus while thorough investigation of animals of herds fitting the clinical syndrome is essential, most herds would be missed if surveillance was entirely based on investigation of high incidence of clinical disease.

It is uncertain as to how long an individual animal may remain a carrier of *M. bovis*, but it should be assumed it may be months (Biddle et al 2003) to years (Bayoumi et al 1988).

Bulk Milk testing via ELISA or PCR is cheap and relatively easy to implement. However, sensitivity appears low (between 6 and 16% in the case of RT PCR), and as yet undefined in New Zealand for the bulk tank milk ELISA. An international study suggests sensitivity for bulk tank milk ELISA of approximately 60% (Nielsen et al. 2015). Thus multiple tests during the peripartum period when likelihood of recrudescence of disease is highest and hence the highest sensitivity is likely to occur, will be required.

Additionally testing of bulk tank milk, or milk from animals under treatment ('hospital' milk) can only assess the status of those animals actually in lactation. Thus any surveillance program must also ensure that nonlactating animals (beef animals and dairy animals prior to 1st lactation) be monitored by a different approach. Use of the serum ELISA appears to be the logical approach, but requires sampling of individual animals which will increase cost.

It appears that the sensitivity of PCR of tonsils is higher than for other tissue samples (i.e. 96% versus 68% per nasal swabs and 19% tracheal swabs; "Epidemiological evaluation of New Zealand Mycoplasma outbreak to 28 Feb 2018"; page 14). Thus any surveillance based on post-mortem samples should include tonsillar crypt sampling. It is possible that surplus calves slaughtered for beef at a young age (ie < 2 weeks) could be included in surveillance. This has the advantage of earlier detection, than where surveillance is carried out in beef animals (at say 2 years of age). While sampling of the tonsils may be feasible in live animals, this is likely to be a invasive, slow and hence expensive and at a significant welfare cost and potentially significant occupational safety and health risk.

Given low sensitivity of all tests, it appears the confidence in defining the status of any individual animal remains low, and that testing strategies will need to be undertaken at herd level.

Results from surveillance during the current incursion suggest that the prevalence of confirmed infection in known trace forwards or trace backs is much higher than where census sampling of the population has been undertaken. This suggests that trace forward, targeted or regional surveillance is more likely to identify previously undisclosed infected herds.

However due to poor recording of livestock movements, particularly of calves, it should be expected that unrecognised animal movements will result in further farms being *M. bovis* positive. Given this, census sampling should continue for five or more years.

The high level of herd and animal movements within the New Zealand dairy industry, and to a lesser extent the beef industry, remains a significant risk factor. Restrictions of movement outside of the specific incursion response would result in economic hardship and restrict the ability of share milkers

and others to grow their businesses. Thus improvements in compliance to NAIT is critically important to ensure that trace/trace backs can be rapidly and cost effectively undertake.

Specific recommendations if eradication is attempted

- A full eradication and surveillance plan needs to be developed and costed.
- The definition of successful eradication, and how it will be measured must be agreed to by all stakeholders.
- The approach will likely differ between the dairy and beef industries.
- Prior to eradication the test characteristics of the bulk milk ELISA and PCR need to be fully described to allow robust modelling of different surveillance approaches.
- Thus the test characteristics of the milk ELISA should be defined at herd level as a matter of urgency.
- Tonsillar crypt vs nasopharyngeal swabs should be collected for PCR from calves and cows slaughtered from known IP's so that the sensitivity of these sampling sites can be defined at animal and group level. Such data will inform subsequent modelling of surveillance both for the beef and dairy industries, and clarify whether nasopharyngeal swabs (which are less invasive to collect from live animals) are sufficiently sensitive enough for surveillance. Presence of lung or upper respiratory tract pathology should be recorded in parallel with the swabs to assess whether improved sensitivity can be achieved by targeting animals with pathology, compared with a surveillance strategy of sampling at random.
- A census of bulk milk samples should be tested by both PCR and ELISA during early lactation for the next five years.
- It is suggested that if the the bulk milk ELISA can be validated in time and it has a moderate sensitivity (>50%), then 4 tests at 14 day intervals be conducted starting at 6 weeks after the start of calving. Milk samples should be held for subsequent PCR testing if any sample is found ELISA positive.
- Given published sensitivity and specificity values for bulk milk ELISA antibody test, four sequential samples per herd will be required to detect all positive herds (Appendix 1). However, it is likely that more than 1,000 false positive herds would be identified, and all of these would need bulk milk qtPCR testing, testing of young stock by ELISA and at slaughter (bobby calves) and further follow up.
- The sensitivity of ELISA testing of animals exposed many months before testing (for example following exposure to infectious milk or in calf rearing sheds) needs to be defined. It is not clear, in the absence of this data, as to what the sensitivity of testing say rising 2 year old dairy replacement animals or beef animals is. Thus the optimal testing strategy for such animals is unclear. Were sensitivity of ELISA used at this age very low, then testing at slaughter (for beef animals) or post calving (for 1st lactation dairy animals) may be the better approach.
- A strategy for dairy farmers to manage importation of service bulls is required. It is likely that testing of individual bulls, even repeatedly, is likely to be of low sensitivity. Thus options include use of AI only, or some form of bull farm assurance of disease status (testing of all bulls prior to sale/lease?).
- Forward trace farms that have not been test positive thus far should have a higher level of scrutiny. Repeated bulk milk testing of these farms the next 5 years should be undertaken.
- Testing capability of all laboratories nationally needs to be improved and clear guidelines provided as to the laboratory's responsibilities where test positive bulk milk's, quarter level milk samples or other tissues are detected.
- Clear lines of reporting and responsibility need to be established as part of any eradication/surveillance plan. Does MPI have enough capability or will experienced cattle practitioners need to get involved (with appropriate training and support)?

Comments and notes from relevant studies

A study of animals entering feedlot in Canada found that 100% of clinically ill and >80% of 'healthy' animals were positive for *M. bovis* detected using either nasopharyngeal swabs or bronchoalveolar lavage (BAL) techniques. Isolation occurred in 100% of the animals for at least 12 days (Allen et al. 1992).

In another Canadian study, *M. bovis* was isolated from nasal swabs of 28/62 yearlings after about four months of introduction to a feedlot, but the prevalence declined with time (Table 2 below). However, only one animal isolated *M. bovis* at slaughter, and this animal had no gross evidence of pneumonia (Yates et al. 1983). There was no association between grossly evident lung lesions and isolation of any pathogen. Due to study design, it is unclear when animals became infected and the duration of infection. However assuming that exposure occurred around time the transport and introduction to the feedlot, the highest prevalence occurred approximately four months later, with nearly half of the animals culture positive, but by six and seven months (i.e. September and October) the prevalence of drop-down to less than 10 and less than 2%, respectively. This suggests that culture at slaughter in animals exposed some months prior to slaughter are likely to have a low prevalence of culture positive, at least in nasal swabs.

Table 1. Microbiology culture results from yearlings (n=62) microbiology sampled at various times after introduction to a feedlot (which occurred in March) Animals were about 11 months of age in March 1981, hence were 12, 15, 17 and 18 months of age in April, July, September, and October , respectively (Yates et al 1983).

TABLE II. Microbiological Isolations from 62 Calves

	Nasal Swabs				Lung
	Pre-transport	Commercial Feedlot			Slaughter
	March 9	April 10	July 15	Sept 24	October 20
P. haemolytica	1	12	8	1	0
P. multocida	0	5	1	12	0
M. bovis	0	17	28	7	1[a]
M. bovirhinis	3	58	26	38	0
Ureaplasma	0	9	8	11	0
M. arginini	2	0	15	2	1[b]
A. laidlawii	0	0	23	3	0

[a,b]Isolated from morphologically normal lung of one animal

An oral challenge model in young calves demonstrated a preference of *M. bovis* to colonise tonsil. It also demonstrated that there was low to undetectable serum antibody concentrations in these animals (Maunsel et al 2012). All eight calves exposed to oral *M. bovis* had tonsils that were heavily colonised and it was suggested that tonsils swabs rather than deep nasal swabs should be used.

In a review, Maunsell and Donovan (2009) noted that there is poor correlation between isolation of *M. bovis* from the upper and lower respiratory tract and poor correlation with clinical disease. They also note that the sensitivity of use of nasopharyngeal swabs at group level has not been determined.

Following challenge, 24 of 24 animals had *M. bovis* recovered from a nasal swab. Following necropsy at 132 days post challenge 23 of 24 lungs with no gross pathology contained *M. bovis*, whereas 24 of 24 lungs with gross pathology also cultured M bovis (Prysliak et al. 2013). This indicates that gross pathology of the lungs is not a good predictor of infection status following M bovis exposure. Hence a strategy of only sampling animals with lung lesions at post-mortem may miss may *M. bovis* cases.

However in a natural exposure study of young animals in Canadian beef feedlots, 99 animals that died or were euthanased within 2 months of entering one of 67 feedlots were examined. Those with gross evidence of lung pathology were about twice as likely to culture *M. bovis* than those without lung pathology (Table 2, from Gagea et al 2006)

Table 2. Culture results by lung pathology (Gagea et al 2006).

	Caseonecrotic bronchopneumonia (n = 24)	Caseonecrotic and fibrinosuppurative bronchopneumonia (n = 30)	Fibrinosuppurative bronchopneumonia (n = 28)	Viral pneumonia alone (n = 4)	No pneumonia (n = 13)
M. bovis	24 (100%)	29 (97%)	23 (82%)	0 (0%)	6 (46%)
M. arginini	22 (92%)	29 (97%)	15 (54%)	0 (0%)	6 (46%)
M. bovirhinis	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)
U. diversum	5 (21%)	8 (27%)	1 (4%)	1 (25%)	0 (0%)

Following intramammary challenge with 10^8 CFU of *M. bovis*, three cows became and remained infected. Two of these animals remained infected through the dry period and into the subsequent lactation (Byrne et al 2005).

In a review of laboratory cases following introduction of *M. bovis* to Ireland in 1993, Blackburn et al (2007) reported that many more herds were defined positive following positive culture of M bovis from calf pneumonia outbreaks (N = 214 herds), in comparison to herds defined as positive based on positive milk culture (n=6) or culture from joint aspirates in lame cattle (n=1). This suggests that passive surveillance of disease in calves may be more sensitive than that focused on mastitis or arthritis in our cattle.

Vertical transmission appears to occur, via placental and colostral transfer (Pfutzner and Sachse 1996). The same authors state that animals infected as calves potentially remain infected through to their first calving.

An Australian study assessed the agreement between PCR, ELISA and culture of bulk tank milk. There was very poor level of agreement between ELISA, PCR and culture. The OD in milk from animals under treatment was higher than that of the main herd (Parker et al. 2017). The highest optical densities were achieved where testing of the bulk milk occurred 5 to 8 weeks after the start of the seasonal calving period, and 5 to 8 months since the initial *M. bovis* outbreak diagnosis (Parker et al. 2017). The bulk milk ELISA ODs decline below the manufacturers' cut-point by 12 months after an initial outbreak.

The sensitivity and specificity of bulk tank milk ELISA reported as 60.4% (95% probability interval 37.5-96.2) and 97.3% (95% probability interval 94.0-99.8; Nielsen et al 2015). This appears substantially higher than the sensitivity as reported within the incursion investigation for bulk tank milk QT PCR (i.e. about 10%).

A US study reported a sensitivity of PCR-based bulk tank milk diagnosis of mycoplasma as 76.7% (95% CI 71-81.3%; Justice-Alan et al 2011), using culture as the gold standard. The authors comment that a number of herds alternated PCR test status across time, which they ascribed to intermittent shedding by individual cows.

A study comparing transtracheal washes, nasal swab, guarded nasopharyngeal swab or broncho-alveoli lavage for diagnosis of *M. bovis* in pre-weaned calves demonstrated greater than 90% agreement, and the kappa was > 0.82 amongst the tests (Doyle et al 2017), with the highest level of agreement being between bronchoalveolar lavage and transtracheal washing. The guarded nasopharyngeal swabs were collected following wiping of nares with a single use paper towel and then advancing a 59 cm swab to approximately 2 cm rostral to the medial canthus of the eye, then

advancing the swab itself a further 4 cm rotating and withdrawing back into the sheath before withdrawal. Swabs were placed in a viral transport media (Eagles minimum essential media with HEPES and sodium bicarbonate with added gentamicin and amphotericin B).

In study of 3 approaches to serology of *M. bovis*, Schibrowksi et al (2018) reported that Western blotting had the highest sensitivity (74% (95% confidence interval (CI): 16–98%)), compared to the BIO K302 (47% (95% CI: 10–87%)) and BIO K260 (28% (95% CI: 1–92%)). However, for specificity the BIO K302 (96% (95% CI: 87–99%)) and the BIO K260 (100% (95% CI: 93–100%)) out-performed Western blotting (88% (95% CI: 56–98%)). Western blotting was the best assay for detecting seroconversion, correctly identifying 61% (95% CI: 29–86%) of exposed animals compared to 35% for BIO K302 (95% CI: 21–54%) and 8% for BIO K260 (95% CI: 0–87%). Samples for this study were drawn from challenge models using different *M. bovis* strains on three continents; Canada, UK and Australia. The final sample time also varied being 68, 28 and 24 days post challenge. There is variation in the proportion that were seropositive at each at the endpoint for the three studies but it is not clear whether this is related to the time between exposure and sampling, or the M bovis strain used. Additionally as the last animals were slaughtered 68 days post exposure, it is not clear when titers wane.

Another study estimated the median Se and Sp of the BIO K302 ELISA to be 60% (38–96 95% Posterior Credibility Interval) and 97% (94–100 95% PCI), respectively, using the manufacturer's recommended cut-off point (Nielson et al 2015).

A further study estimated that the bulk tank milk the sensitivity of the Bio K 302 ELISA in bulk tank milk was 43.5% (95% CI: 21.1-92.5%), and specificity equals 99.6% (95% CI: 98.8-100%; Arede et al 2016). Using this ELISA and undertaking a census of dairy herds in Denmark, the apparent prevalence ranged between 1.5 and 5.2% of herds. For census cross-sectional surveys were taken over one year and based on the geolocation and ELISA data demonstrated that high risk clusters occurred, but appeared to have a short duration.

Appendix 1. Simple model of sensitivity and specificity and number of false negative and false positive herds following census testing of bulk milk samples for all New Zealand dairy cows on multiple occasions, with interpretation in parallel. The sensitivity and specificity are derived from the bulk milk ELISA estimates from Nielsen et al 2015. For the sake of this modelling, it is assumed that the true prevalence is 30 herds nationally.

Se	0.604
Sp	0.973
no. herds	12000
Prior prevalence	0.0025
num true +ve herds	30
num true -ve herds	11970

					Herds:	
no. tests	SE	SP	PPV	NPV	Num false -ve	Num false +ve

	1	0.604	0.973	0.0531	0.9990	11	324
	2	0.843	0.947	0.0382	0.9996	4	638
	3	0.938	0.921	0.0290	0.9998	1	944
	4	0.975	0.896	0.0230	0.9999	0	1242

Sample size calculations for serum ELISA
http://epitools.ausvet.com.au/content.php?page=FreedomFinitePop&Population=100&SampleSize=300&Sens=0.5&Conf=0.95&Prevalence=0.02
Assuming that the antibody prevalence is ≥10% if *M. bovis* is present in a group of animals, and that the groups size is 100 and that we want to be 95% confident that the prevalence is >10% with a test with a 50% sensitivity, then we need to sample 60 animals.

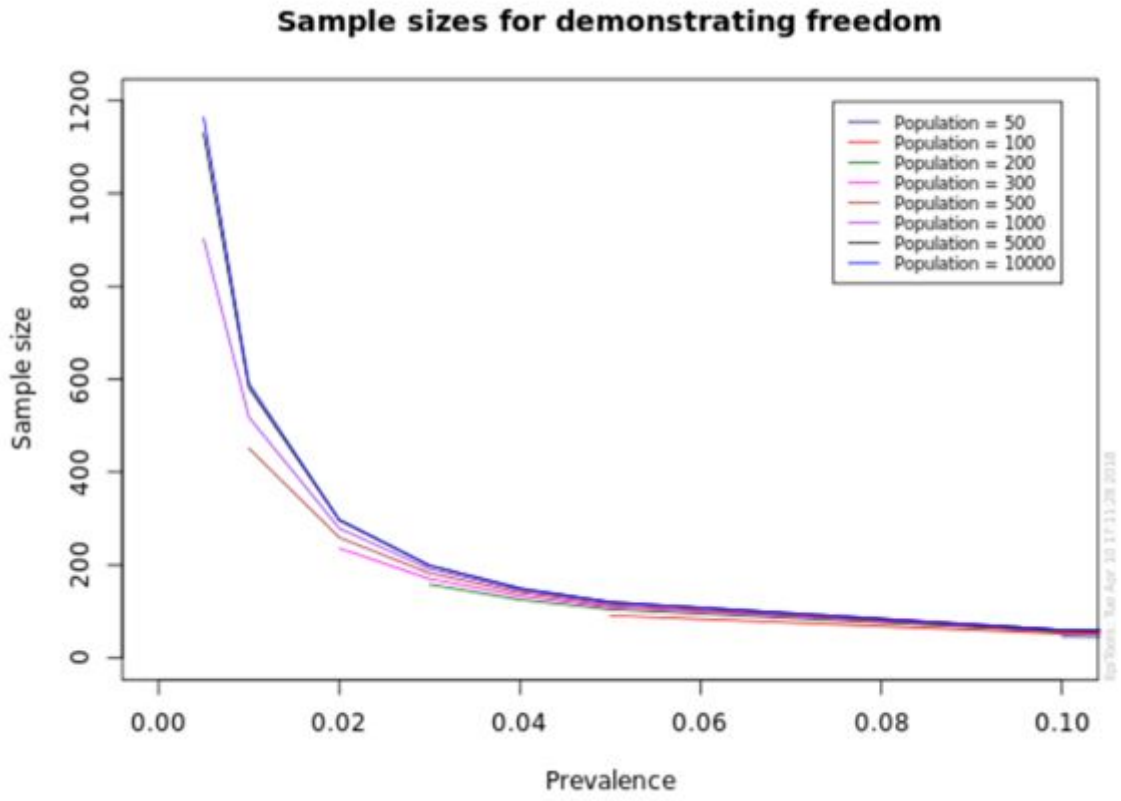
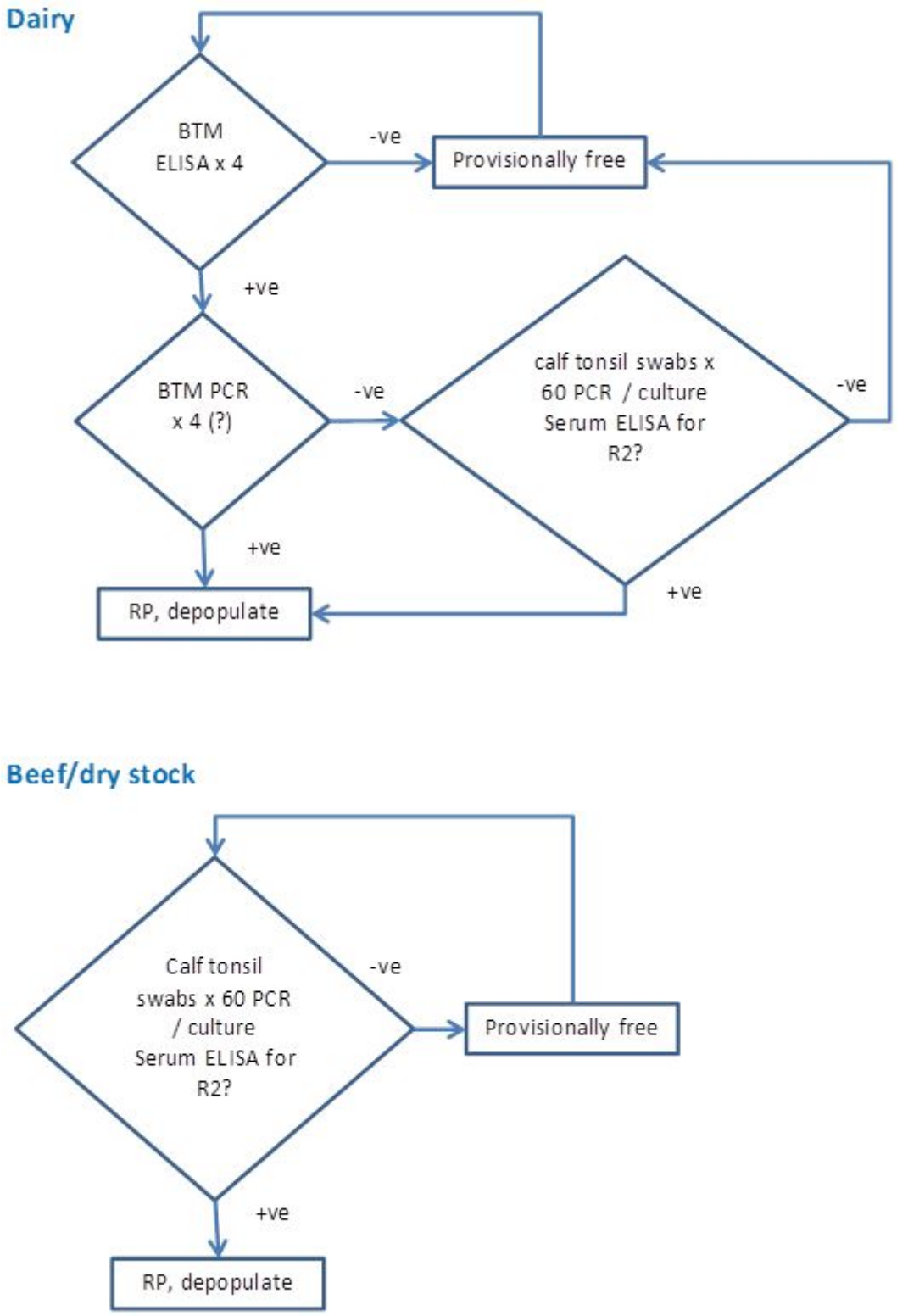


Figure 1. Simple schema for potential surveillance of dairy and beef farms



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Question 5) Persistence in the environment

- Provide a scientific assessment of the cleaning, disinfection and stand-down protocols for decontaminating infected properties prior to repopulation.
- Advise on whether changes could be made to expedite the process without unduly increasing the risk of disease transfer to new stock.

The proposed cleansing, disinfection, and stand-down protocols appear thorough and consistent with international best practice. Reflecting the general fragility of *Mycoplasma bovis* and its susceptibility to disinfectants, the proposed two stage disinfection process may be unnecessary. Reducing the protocol to include a single disinfection step is unlikely to significantly increase the risk of re-infection.When decontaminated farms are repopulated with cattle, these cattle should be frequently tested and it should become quickly apparent if the decontamination was ineffective. Such decontamination should be applied to both infected properties and all transport vehicles after use.

The lack of a cell wall renders mollicutes fragile although they are able to produce biofilms which may enable their survival in the environment making them more resistant to heat, desiccation and oxidative stress (McAuliffe et al., 2006). Because of biofilm formation, *M. bovis* can survive for nearly 2 months in sponges and milk; and for over 2 years in water, although at higher temperatures survival rates drop considerably. *Mycoplasma bovis* was detected on the pens of infected white veal calves in Italy (Piccinini et al 2015) though whether there was sufficient mycoplasma there to initiate a respiratory infection is debatable. It is known that in their biofilm state the mycoplasmas overexpress certain proteins associated with pathogenicity factors possibly making them more virulent (McAuliffe et al 2008). The close similarity between *M. bovis* and the small ruminant pathogen, *M. agalactiae*, the cause of mastitis and other conditions (contagious agalactia) also known as “mal di sito” because of its apparent persistence on a farm, suggests *M. bovis* may possess similar properties. Few mycoplasmas, possibly enough present in bedding contaminated with infected milk, are necessary to initiate an udder infection in ewes. As yet, no evidence has been produced to confirm these finding for *M. bovis*.

Mycoplasma bovis can survive in materials found in a cow’s environment when these materials become contaminated with naturally infected milk (Ruffo et al., 1969). When such milk was applied to sterilized material normally found in a dairy barn it was found that manure supported the growth of *M. bovis* for 37 days at 20°C yet would only survive for 24 h on wood surfaces (Ruffo et al., 1969). Survival of *M. bovis* on environmental surfaces is affected by temperature; viability was best at 20° and recovery of organism begins to decline at 37°C (Ruffo et al., 1969). Absence of light was found to best support pathogen sustainability. Sunlight reduced the survival of the microorganism in the aforementioned materials with the exception of rubber. A rubber surface supported maintenance of *M. bovis* at the same level for 4 days at 23-28°C either in sunlight or dark (Ruffo et al., 1969).

Mycoplasma bovis infected milk was contaminated with the materials described above but the materials were in their “natural conditions”, not sterilized apriori (Ruffo et al., 1971). *Mycoplasma bovis* for a second time was found to survive longest in manure, where the microorganism could be

isolated 236 days after contamination (Ruffo et al., 1971). Least persistence was associated with wood, from which *M. bovis* could not be re-isolated after 24 hours. Survival in water contaminated with infected milk was tested and *M. bovis* was capable of surviving for 23 days at 23-28°C when not directly exposed to sunlight (Ruffo et al., 1971). Survival times were remarkably reduced when the temperature was at 37°C versus 20°C and when the materials were exposed to sunlight (Ruffo et al., 1971).

Thus *M. bovis* can survive for at least 236 days under dairy like environmental conditions. Justice Allen et al. (2010) reported that *M. bovis* could survive in dairy bedding, recycled sand, for at least 8 months. Similarly to Ruffo et al. (1969, 1971) they (Justice Allen et al. 2010) reported that temperatures of 15-20℃ best supported viability. They also reported that 0.5% sodium hypochlorite or 2% chlorhexidine were effective in eliminating *Mycoplasma sp.* from contaminated bedding. This suggest that simple disinfection of dairy surfaces and the environment can be used to eliminate the agent. However, aerosol transmission has been proposed as a mechanism of spread of this agent from infected and colonized cows to naïve animals. *Mycoplasma sp.* have been recovered from Petri dishes exposed to barn air (Jasper et al., 1974).

Despite the ability of the pathogen to remain resident in the dairy environment, it is considered unlikely that the environment provides a major source of infection. More generally, Mycoplasmas are readily killed by disinfectants and do not survive for prolonged periods outside the host. There is a vast amount of evidence in published literature to demonstrate the environmental fragility of *Mycoplasma* spp.

In poultry, *Mycoplasma gallisepticum* remains viable in chicken faeces and on cloth for up to 3 days at 20°C, in egg yolk for 18 weeks at 37°C or 6 weeks at 20°C, and in distilled water for 24 hours at both 4°C and 22°C. *Mycoplasma iowae* has been shown to survive for 5 days or more on feathers and at least 6 days on human hair, cotton, rubber, and straw. *M. iowae* appears to be slightly hardier in environmental conditions than other mycoplasmas although the organism appears to be inactivated by proper cleaning and disinfection.

Several OIE-Listed diseases are associated with Mycoplasma spp. including contagious bovine pleuropneumonia (CBPP) (*Mycoplasma mycoides* subsp. *mycoides* (Mmm), avian mycoplasmosis (*Mycoplasma gallisepticum*), contagious agalactia (*Mycoplasma agalactiae*) and contagious caprine pleuropneumona (CCPP) (*Mycoplasma capricolum* subsp. *capripneumoniae*). The OIE technical disease cards for CBPP and CCPP both note that the causative organism is very fragile and not able to exist long in the external environment. On average the organism only survives outside the host for 3 days in tropical areas and up to two weeks in temperate zones. The OIE also note that the organisms are inactivated within 60 minutes at 56ºC and within 2 minutes at 60ºC.

Iowa State University Center for Food Security and Public Health state that alcohols, aldehydes, alkalis, biguanides, halogens, oxidising agents and phenolic compounds are ‘highly effective’ against *Mycoplasma* spp., whilst acids and quaternary compounds are considered ‘effective’.

A search using the Web of Science with the terms TOPIC: (Mycoplasma) AND TOPIC: (effluent OR manure) AND TOPIC: (persistence OR survival), failed to locate any articles on mycoplasma survival in effluent.

ADDENDUM

Subsequent to the final TAG teleconference, MPI informed TAG that current cleansing and disinfection requirements were not working well. Considerable effort has been spent cleaning equipment that has had limited contact with animals. Cleaning of yards and buildings has been extremely time-consuming, with it taking more than 60 days on some properties to complete two rounds of cleaning including the two-week drying period, after which time the 60-day stand-down begins. In practice therefore, farms are experiencing a stand-down in excess of 120 days. This is

delaying farmer repopulation plans, and imposing unforeseen costs on farmers and (via compensation) MPI.

MPI has therefore proposed changes largely around when the 60-day stand-down period before repopulation begins.

- Pasture requirements above will be similar/the same, but the 60-day stand-down period will begin when the last cull animal leaves the farm
- Zoning of farms will be permitted, with animals potentially moving back onto pasture before being allowed into higher-risk areas such as buildings
- Clearer instructions around prioritising cleaning of high-risk area, based on the amount of animal contact and the ability of animal secretions/waste to accumulate
- Moving to a single round of cleaning and disinfection, on the “Do it once - do it right” principle
- There is debate over whether the 60-day stand-down period for buildings/yards should begin when the last animal leaves the farm, or when the cleaning is complete.
- If cleaning and disinfection is effective, no stand-down period at all should be required.
- Given the inability to be confident all bacteria have been killed, a stand-down period of any length allows some margin for error.
- In essence, the debate is over how risk-averse MPI should be. Advocates for a shorter period note that there is negligible evidence of spread via fomites in the New Zealand outbreak to date.
- One of the most expensive aspects of cleaning and disinfection has been the removal and disposal of effluent to land fill, up to 100k per property. Disposal by burial on farm in the wetter months of now to next spring are unlikely to be practical, due to a rising water table. The option to treat the pond by spraying an acidic liquid over the surface to inactivate mycoplasma and let the rest be inactivated by anaerobic composition would be much cheaper, if it can be left on farm. It is theoretically possible to inactivate mycoplasma in this way but it has not been tested.
- Are there any other practical options for effluent disposal that do not require transport of this material off farm?

MPI’s observations regarding the challenges associated with cleansing and disinfection of properties are consistent with experience overseas, for example, during the UK FMD outbreak of 2001. However, in the case of *Mycoplasma bovis*, there is no published evidence that indicates the environment should be considered a significant source of infection. Whilst there is little evidence that will allow the above questions to be answered directly, beginning the 60 day stand down period when the last animal leaves the property and relying on anaerobic inactivation of the organism in effluent ponds is unlikely to result in a significant increase in the likelihood of re-introduced stock being infected from the farm environment.

However, reflecting uncertainty associated with this procedure (no other country has tried to eradicate *Mycoplasma bovis* from a farm environment), it would be prudent to fully restock properties only after sentinels have been introduced and tested for infection. As noted above, when decontaminated farms are repopulated with cattle, these cattle should be frequently tested and it should become quickly apparent if the decontamination was ineffective. However, it is considered unlikely that the environment provides a major source of infection.

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