Technical Advisory Group Report to the Ministry for Primary Industries on Potential for Eradication of *Mycoplasma bovis* from New Zealand

December 2017

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Executive Summary

From the incursion investigation of *Mycoplasma bovis* undertaken between July 22 and December 11 2017, the weight of evidence favours the conclusion that there has been a recent point source incursion of *Mycoplasma bovis* into New Zealand. Currently all infected places appear to belong to either a single cluster, or possibly two clusters, with transfer of infection within clusters largely linked to animal movement.

The mechanism by which infection was introduced, and a possible source country or region, have not been determined. Several possible entry mechanisms have been postulated, but all are considered unlikely, and there is insufficient evidence to rank them in order of likelihood. Further investigation of likely pathways is needed, as the likelihood of a further incursion in the near future is a factor that should be considered in the decision on eradication.

Given current understanding of the epidemiology of the disease, and provided that the ongoing delimiting survey work does not identify additional unrelated clusters, eradication appears feasible. A decision not to eradicate, and to allow the disease to become endemic, would be likely to lead to substantial on-farm costs, and increased antimicrobial use.

The prospects for successful eradication will be most favourable if it proceeds rapidly. Given the large number of animal movements associated with the end of sharemilking contracts on 31 May, national eradication should be complete by no later than mid-May, or at least any remaining infected or suspect herds should be under strict movement control by then. The prospects of achieving this will be greatest if all infected places are identified and movement controls applied by mid to late February, so that a final decision on whether to proceed to eradication can be made before the end of February.

Bulk milk testing is an important tool for rapid pre-decision surveillance. It is noted that the qPCR may have lower sensitivity than serology for detecting infected herds. However, concerns about the low specificity of the currently used ELISA, with the likelihood of a large number of false positive herds, is currently precluding its use as a bulk tank milk screening test. Further optimisation of the ELISA testing procedure (specifically reassessment of the level of optical density (OD) that is used as a cut-off point) should be undertaken in order to develop an optimal testing regimen.

Further proof of single point source of the current incursion would be provided by demonstration that the *M. bovis* from multiple infected properties (IPs) are of the same genotype. Thus isolation of the organism and whole genome sequencing of isolates from IPs outside the s 9(2) herds is strongly encouraged, and this should be completed before the final decision about whether to proceed to eradication.

Specific recommendations

- Undertake progressive qPCR evaluation of bulk tank milk and discarded milk from all dairy herds, commencing in the lower half of the South Island. If all herds in this region other than known IPs are found to be negative, expand testing throughout the country until all herds have been tested nationwide.
- If any herds test positive in this qPCR evaluation, investigate urgently, in order to facilitate a decision on whether to proceed with eradication.
- Refine the ELISA test, if possible, and determine whether it can replace or supplement the qPCR assay in some parts of the testing program, without loss of diagnostic accuracy.
- Investigate s 9(2) to identify likely sources of infection, and whether it is epidemiologically linked to the s 9(2) group of herds. Give top priority to isolating *M. bovis* from this herd, and use MLST and whole genome sequencing to determine whether the same strain is present on both s 9(2) and s 9(herd complexes.
- Investigate possible entry mechanisms for the incursion in greater depth, especially the alternative hypotheses put forward by the TAG as a result of information gathered during the TAG meeting.
- Prepare a policy assessment by the end of February 2018, evaluating the relative epidemiological and economic merits of eradication versus alternative control strategies outlined in this report, and any other strategies that appear appropriate in the light of findings emerging from the investigations in the above recommendations.
- Resolve major deficiencies in the practical operation of the National Animal Identification and Tracing system (NAIT) and in information systems available to the Ministry for Primary Industries for managing incursions and control activities.
- Aim to complete eradication by mid-May 2018, if that is the chosen strategy.
- Maintain national surveillance for 3 to 5 years after completion of eradication, to detect any emergent foci of infection, which are most likely to arise from animals infected during the rearing phase, before first lactation.
- As part of this surveillance, ensure that the risk of infection being maintained in animals born in infected herds but not yet lactating is given adequate attention within the post-eradication surveillance program, including the possibility that they may infect animals with which they are co-mingled during rearing.

Epidemiological assessment

The index farm (s 9(2)) still appears to be the best candidate for the primary case, where the incursion began. However, if this is true, then there must be an explanation for how s 9(2) became infected from the s 9(2)(b)(ii) group of herds, or one of the other IPs. This requires intensive investigation, together with an assessment of whether there is a possible undetected primary case that precedes both of these infection clusters.

s 9(2 showed an extreme outbreak form of the disease, also seen overseas, that strongly suggests a point source event, in which many animals became infected over a short period, rather than a propagating outbreak, in which infection was transmitted progressively through the herd. There were incidents of enteric disease in calves and pneumonia in adult cows that preceded the main disease outbreak, but their relevance is uncertain and their relationship with the *M. bovis* incursion is not supported by any laboratory evidence. The main disease outbreak appears to have begun with multiple cows being affected with arthritis of a single foreleg fetlock joint in all cases, an unusual manifestation, although polyarthritis is a feature of the disease.

s 9(2)(g)(i)

A large number of cows that were due to calve in July were then noticed to have swollen udders, suggestive of premature onset of lactation, 2 to 4 weeks early. Examination of the affected cows showed that they had atypical mastitis in all four quarters, unlike any cases seen in the area previously. When the cows eventually calved, many of the births were premature by about two weeks, and the calves showed poor viability, with a high case fatality rate over the early weeks of life.

s 9(2 received 35 lactating cows from a linked farm, s 9(2 , in May for a two month period. When these cows were returned to s 9(2 it precipitated an outbreak of serious, but milder, disease in this herd. A small number of additional exposed herds developed milder disease, while other herds that tested positive had no clinical disease reported. This pattern of variation in severity of disease is typically seen in *M. bovis* outbreaks.

TAG TASK 1-Testing and Surveillance

- ***Provide an evaluation of the surveillance programme, including:***
  - ***The epidemiological and diagnostic methods used,***
  - ***The interpretation of surveillance data,***
  - ***An assessment of the remaining uncertainty about whether* M. bovis *is present but not detected elsewhere in the country,***
  - ***Recommendations for a suitable testing regimen for declaring a group of animals "negative for* Mycoplasma bovis*" and,***

- ***The confidence that that regimen would provide around a declaration of negativity.***
- ***Recommendations for a suitable testing regimen for declaring a group of animals negative or positive for* Mycoplasma bovis*, and the confidence that that regimen would provide around a declaration of negativity or positivity for restricted places.***
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Evaluation of diagnostic methods used in the surveillance program

The samples used for the initial identification of *M. bovis* were received on 20th July, 2017. Preliminary results were provided on 21st July and the diagnosis was confirmed on 22nd July, indicating that the laboratory was well-prepared for making a diagnosis with a short processing time.

The surveillance programme has relied on a combination of five diagnostic methods, with choices to use each of these methods dictated by availability and changing circumstances over the course of the response. It is the opinion of the TAG that the choice of diagnostic methods and their application at different stages of the response has been entirely appropriate and highly effective, given both the known limitations of each of the methods and the uncertainty about their application in surveillance in an apparently previously uninfected population, unlike populations investigated in other parts of the world.

The methods used during the incursion include:

- Conventional polymerase chain reaction (PCR) assays on milk and/or tissue for detection of *M. bovis* DNA;
- Real-time or quantitative polymerase chain reaction assays (qPCR) on milk and/or other samples for detection of *M. bovis* DNA;
- Microbiological culture for detection and subsequent identification of *M. bovis*;
- Enzyme-linked immunosorbent assays (ELISAs) for detection of antibodies against *M. bovis*;
- Multi-locus sequence typing (MLST) for characterisation of strain variation among *M. bovis* isolates;
- Whole genome sequencing for characterisation of strain identity and diversity among *M. bovis* isolates.

The only methods suitable for large scale surveillance of potentially infected herds and animals are the PCR assays and ELISAs.

PCR assays

The qPCR assay has an apparently high but imperfectly defined sensitivity and very high specificity. The conventional PCR assay has similar sensitivity but much lower initial specificity, due to its limitations in differentiating different mycoplasma species. This limitation was addressed during the early phase of the investigation by sequencing the PCR

product from any suspect positive cases to ascertain whether the assay had detected *M. bovis* or some other mycoplasma species.

Conventional PCR was used initially as this was available in the laboratory, but as sample throughput increased and higher laboratory capacity was required, the laboratory switched, after appropriate testing to ensure maintenance of sensitivity, to a qPCR assay that had very high specificity coupled with high throughput DNA extraction methods.

Antibody assay

None of the currently available commercial ELISA kits have been well validated by the suppliers in terms of sensitivity or specificity, particularly for serum samples. The laboratory identified the best of the currently available assays and ensured an adequate supply of these kits from Canada for the surveillance programme. The interpretation of the results from these assays has been appropriate given the uncertainty about their sensitivity and specificity. The data now available on the performance of this assay under New Zealand conditions can be analysed to identify an appropriate cut-off value for application in future eradication or containment programmes. While this assay has thus far been applied to serum samples, the additional evidence concerning its diagnostic accuracy may potentially be applied to facilitate bulk milk sample testing in the future, subject to appropriate initial validation.

Culture and genetic characterisation

The laboratory has, appropriately, used culture of the organism to only a limited extent so far because this is much more laborious and time consuming to undertake than the other diagnostic methods available, and thus unsuitable for a rapidly changing situation. However, it allows genetic characterisation to be conducted, which is essential for evaluating the epidemiological linkages between different infected places (IPs). So far it has been used to recover a series of 16 isolates from s 9(2, which have then been subjected to strain typing using both MLST and whole genome sequencing. All isolates were clonal, indicating a lack of genetic variation on this farm, consistent with a recent infection, derived from a single source.

It has not yet been used on any other premises. Further culturing and genotyping of isolates from other confirmed infected properties (especially s 9(2)) is therefore now a high priority task, to support, or refute, the hypothesis that a single clone is responsible for disease on all known infected properties, and hence whether the incursion is recent and had a single entry mechanism, or is of longer duration and/or results from multiple incursions.

Preliminary sequencing data have also enabled some comparisons to international strains of *M. bovis*, to help narrow the options for the possible source of the strain and its mechanism of entry. While the limited sets of data available in both the MLST databases and the whole genome sequences in Genbank restrict the conclusions that can be drawn, preliminary comparisons suggest that the NZ isolates from s 9(2 form a distinct clonal group that appear on the sparse available evidence to be more closely related to European or North American strains than to Australian or Asian strains. At present the low numbers of characterised international strains preclude any specific attribution of the source of the New Zealand *M.*

bovis incursion. The surveillance team has been appropriately circumspect about interpretation of these data.

The rapid increase in demand for laboratory services (considerably more than double normal throughput over the last 6 months) has been met through a variety of short term measures, but any continuation of demand at this level, or any increase in demand, will require significant investment in obtaining access to additional highly-trained personnel capable of carrying out the specialised laboratory techniques required.

Epidemiological investigation

Investigations in the delimiting phase are continuing. Prior to the commencement of the TAG meeting, the available surveillance data strongly suggested a recent incursion and a single cluster of cases. During the course of the TAG meeting, new information, which was not then publicly available, was provided to the TAG, concerning two new foci of infection. The first of these is geographically significant because it is located at Hastings, which means that infection is present in the North Island as well as the South Island. However, it was a direct animal movement trace from the s 9(2) complex, and therefore it may not represent any change in the epidemiological situation. The second new focus is s 9(2), which is a group of 3 s 9(2) herds in the Winton area of the lower South Island. While there are plausible reasons for an epidemiological link to the s 9(2) complex of herds, the investigation is at too early a stage for evidence to be available on this point. Crucial questions about s 9(2) are whether infection preceded that in s 9(2) temporally, or whether infection in this group of herds is linked to the infection in s 9(2) as a secondary case, or to another as yet unidentified earlier infected herd within the s 9(2) group or elsewhere. If s 9(2) became infected before s 9(2), this will lead to a detailed investigation of entry mechanisms that could explain a possible incursion resulting in infection of s 9(2) (if it is the primary case), or further back-tracing if it is a secondary infection derived from a currently unidentified primary case. Thus, very detailed investigation of s 9(2) and movements of possible vectors of infection into s 9(2) is a top priority task.

Progress of scanning and targeted surveillance

Since July there has been a major commitment of resources to sample the cattle population (Table 1). Using multiple surveillance strategies, the investigations have yielded entirely negative results.

Table 1 Volume of surveillance testing between July and December 11, 2017

Surveillance method	Response area surveillance (regional)	Massey survey of high risk dairy farms (national)	High risk beef farms	Routine mastitis milk samples submitted to commercial labs (national)	Total
PCR (milk/swab)	8213	878	2851	4973	16915
Serology	35455	714	2059	-	38228

(serum)					
Total samples (% of total samples)	43668 (79%)	1592 (3%)	4910 (9%)	4973 (9%)	55143

This scale of sample collection and testing has required the marshalling and management of all available human resources by the laboratory, made more difficult by the fact that staff are operating in temporary facilities while the new laboratory building is under construction. It has also required a major commitment to field sample collection and coordinated effort between the laboratory and field investigators, with multiple sample collection methods being used.

The TAG considered the use of multiple methods of sampling the cattle population (both dairy and beef), and the large scale of the sampling, to have demonstrated that infection is not endemic in the New Zealand cattle population, and that any as yet undetected foci of infection will be components of specific epidemiological networks. Characterisation of the linkages within such networks by intensive investigation should provide leads on the method of introduction of the organism, and factors influencing its persistence and spread. The identification of s 9(2) by targeted surveillance represents an important step in resolving how many infection networks exist, and what causal and contributory factors are involved. s 9(2) in Hastings has a previously identified link to the s 9(2) complex, which adequately explains its infection status, and the requirement in that case is to determine whether any onward transmission in the North Island has occurred.

Further surveillance in the response phase, through testing bulk tank milk and pooled discarded cow milk in both North and South Island dairy farms is now required to detect any cryptic foci and hence to comprehensively assess the spread of the incursion. This will help to determine the composition of eradication or control strategies.

Recently exposed herds are expected to be immunologically naïve and therefore have higher incidence of clinical disease, detectable infections and infection prevalence at present. In addition to the clinically affected animals, it is expected that a significant percentage of the herd population was exposed to the agent and became subclinically infected. Thus in the near future the number of cattle that were previously infected and that are now seropositive will be higher, while indicators of active infection are expected to be lower. Thus it is expected that detection of disease by qPCR will be easier in herds that are closer to the initial incursion than later in the incursion, when qPCR prevalence may be lower, but seroprevalence and milk antibody prevalence higher. However the low sensitivity of the current ELISA means that the true prevalence of antibodies may be under-estimated, and some herds may be incorrectly assessed as negative if only the ELISA is used. Specificity is also lower than desired, which means herds which are found to have low seroprevalence and low or zero qPCR positives are likely to be uninfected. Thus interpretation of ELISA test results is challenging for deciding the infection status of a herd in which apparent prevalence does not clearly classify the herd as positive or negative, because both the sensitivity and specificity of the test are lower than needed. In this type of situation, confirmatory tests applied either in serial or in parallel are

typically used to resolve uncertainty about the status of the herd. It may be desirable to develop an immunoblotting test to meet the need for a confirmatory test.

When using qPCR to identify the presence of infection in a herd, consideration must be given to fluctuations in shedding of organisms, which can result in false negative diagnoses. Currently, three herd tests are being conducted before a potentially exposed herd is declared negative, as a valid way of minimising the risk of false negative diagnoses, with an interval of at least three weeks between tests. While conclusions could be reached faster with a shorter inter-test interval, TAG supports the current testing procedure for declaring herds negative, because it is important to have a high level of assurance when potentially exposed herds are declared negative, if eradication is to be successful.

Evaluation of an RP to define whether it is an infected place or not

Based on the limited number of herds so far investigated, infected premises in NZ have had high infection prevalences (typically over 30%) that are presumed to reflect recent infection of naïve herds, whereas herds that had test prevalences of under 5% were classified as uninfected despite some positives, reflecting the effect of low test specificity .

This high seroprevalence should be used in development of the investigation strategy. This is based on 3 assumptions: (a) individual animal titres are likely to remain high for months after exposure; (b) herd serological prevalence is likely to remain high for months following exposure; and (c) the clinical disease syndrome, although variable, does not affect the required sample size because the declaration of negative herd status depends on laboratory testing, not clinical signs. On this basis, the minimum seroprevalence to declare a herd infected has been set at 10%, and this is endorsed.

This assumption is critical to the testing strategy described below, and should be evaluated as additional test results become available.

Milking herds

Initial screening should be conducted on bulk milk and/or waste milk (milk withheld from the supply). If the result is qPCR positive at a suggested Ct $\leq$35, the herd will be defined as suspect positive. One or more confirmatory tests should be undertaken - with at least one more positive result - before confirming the herd as an IP.

In order to declare an at-risk herd free of infection, bulk milk or waste milk should test qPCR negative on one or more occasions, depending on the level of risk assigned to the herd. Serology should be then undertaken if a declaration of freedom is needed in the particular situation. The sample size required to detect infection is suggested to be 60 animals, assuming a test sensitivity of 50%, a 95% confidence level and a commercial size cow herd (epitools.ausvet.com.au). Three tests with <5% ELISA test positive (ideally at times of high cow stress such as calving) followed by a final negative qPCR test of bulk tank and waste milk would result in a declaration of disease freedom. Where herd ELISA seroprevalence is >5% at one or more tests, additional epidemiological assessment of the herd will be required, involving additional testing. Depending on the herd structure and suspected introduction route, there is a possibility that only some cohorts of animals on the farm may be

exposed. In such cases these at-risk cohorts may need to be the principal focus of testing activity.

For IP or RP herds where the first rounds of testing indicate that seroconversion is currently occurring, slaughter of the entire herd or an epidemiologically distinct cohort should be considered. Tonsillar crypt sampling for qPCR or culture, as well as culture or PCR of any lung lesions should be undertaken where appropriate to clarify the situation. Use of the tonsillar crypt as a source for qPCR samples is a new approach, and it would be beneficial to confirm its suitability in a comparison with more traditional samples from sources such as lung.

Non-milking herds

Where a herd from which it is not practical to collect milk samples (such as a beef herd or a herd of maiden heifers) is considered at risk based on an epidemiological risk assessment, serology on a sample of the herd as described above should be undertaken. If the seroprevalence is below 10% at each test, the herd can be declared negative. If the seroprevalence is 10% or above, then qPCR (and possibly culture) should be undertaken on samples appropriate to the particular herd type, such as lung or tonsillar crypt. Herd replacements raised in heifer rearing operations which may have permitted them to be exposed to cohorts from other dairy operations where M. bovis was present should be tested by ELISA prior to exit from these rearing operations.

TAG TASK 2 Entry pathways

- ***Provide an official review of the pathways analysis report,***
- ***Considering the pathways analysis report and other material provided by the response team, provide assessments of:***
    - ***The level of uncertainty around correctly identifying the entry pathway,***
    - ***Whether this outbreak is the result of a recent incursion of* M. bovis *into New Zealand,***
    - ***Whether a further introductory event is likely to occur again, and***
    - ***Whether the index farm for this outbreak was the primary farm.***

Review of the pathways analysis report

The private veterinarian who provides services to the s 9(2) herds recognized the unusual nature of the disease syndrome she was seeing, and pursued it vigorously to the point where she reported it as a possible incursion of an unwanted organism, a conclusion which proved to be correct. During the TAG meeting she provided valuable insights into what had occurred, which helped TAG members identify issues which deserved active investigation earlier in the incursion response, and should now be followed up promptly.

The TAG would have expected to receive during its meeting a more detailed description of clinical, epidemiological and pathological findings from the property investigation conducted in the early stages of the incursion response, and especially a comprehensive evaluation of all possible routes of entry of infection to s 9(2, as distinct from a more general assessment of routes by which infection might enter any farm in New Zealand. This would be derived from interviews with people associated with the property and data on movement of people, animals and fomites that might have been related to the incursion. The entry pathways report would have benefited considerably from incorporating this evidence, some of which was drawn out during the TAG meeting as a result of questioning of people by the TAG. Powers are available under the Biosecurity Act to allow all of the necessary investigations to have been conducted on s 9(2, and if there were factors which inhibited investigators in using the powers, they should be brought out and any necessary adjustments made to procedures. The possibility of environmental factors or unwanted organisms other than *M. bovis* being contributors to the various syndromes seen on s 9(2 also seems to have received limited investigation.

MPI's rapid risk assessments for *M. bovis* in bovine semen s 9(2)(a), 2017a), in bovine embryos s 9(2)(a) 2017b), and in non-bovine species (s 9(2)(a), 2017c) provide robust assessments of the risks posed by these commodities. These reports clearly identified areas of uncertainty, indicated clearly where assumptions were made, and provided an objective and transparent assessment of risk, consistent with recognised risk analysis procedures. All three documents presented an exhaustive review of available relevant literature.

The TAG was also presented with a rapid risk profile for *M. bovis* in bovine feed, used equipment, s 6(c) (s 9(2)(a), 2017). This document did not conform to a conventional risk analysis structure, discussed risk in very general terms, and added little useful information germane to this outbreak. This document concluded that current import health standards and border control measures were sufficient to manage the risk associated with these commodities, s 6(c)

More in-depth on-farm exploration of the possible routes of introduction and better communication of this to the risk team would have been likely to have resulted in a much more focussed and informative report.

The TAG were concerned by the limited depth of investigation in the risk pathways report (s 9(2)(a) *et al*, 2017) that accompanied these risk assessments, especially the summary presented as Figure 4 of this report. Further questioning indicated a subjective process had been used to generate this semi-quantitative assessment. The World Organisation for Animal Health (OIE) is very clear that semi-quantitative methods are not suitable for risk assessment (OIE 2010). Moreover, the shortcomings of a semi-quantitative approach was one of the arguments previously used by New Zealand in their successful WTO case regarding export of apples to Australia (MFAT 2008).

Considering the pathways analysis report and other material provided by the response team, provide assessments of:

The level of uncertainty around correctly identifying the entry pathway

"When you have eliminated the impossible, whatever remains, however improbable, must be the truth"

Sir Arthur Conan Doyle

In the case of this *M. bovis* introduction, all the entry mechanisms that can be postulated are improbable, yet one of them resulted in entry. This is a common feature of past disease incursions, which have occurred despite strong border protection, in that the confluence of multiple rare events precipitates establishment of infection. In such circumstances, it is wise not to rank alternative explanations in terms of likelihood, but to pursue all possibilities equally.

To date, MPI investigations have focussed on imported semen as the source of *M. bovis* s 9(2)(a) . Despite MPI's comprehensive review of available literature (s 9(2)(a) 2017a), there is no demonstrated evidence that transmission of *M. bovis* through naturally infected frozen semen can occur. Whilst this does not indicate that this route is associated with 'zero risk', the TAG has identified a number of additional hypotheses that might also explain the introduction of *M. bovis*. There were divergent views within the TAG regarding the likelihood of each of these pathways, although there was a consensus that investigations needed to carefully consider all possibilities beyond the current focus on imported bovine semen.

Semen imports

This has been the primary focus of MPI as the source of this outbreak to date. New Zealand has a long history of importing around 250,000 straws of semen each year for several decades. If semen was likely to be a pathway for the introduction of *M. bovis* then it would be reasonable to expect this organism to be endemic, and widespread in the country. Surveillance described above, plus earlier surveys, have shown clearly that infection is not ubiquitous in the New Zealand cattle population. It could be argued that, because the only specific provision against *M. bovis* in the Import Health Standard for bovine semen entering New Zealand is the requirement that "donors have never recorded a positive test for *Mycoplasma bovis*," that there is no specific requirement that testing be conducted on the bulls. The antibiotics used in preparing frozen semen for export to New Zealand provide another component of the procedures used to protect against entry of unwanted organisms.

New Zealand has been a fifty year field study to show that the introduction of this organism is unlikely to occur through the use of imported semen. The antimicrobial susceptibility profile of the strain of *M. bovis* isolated from s 9(2 does not indicate that it is a resistant strain, so it is unlikely that this organism would be resistant to the antimicrobials routinely added to semen extenders.

There is limited evidence demonstrating the pathogenicity of *M. bovis* for the reproductive tract under experimental conditions, but no evidence obtained under field conditions. Positive PCR results were obtained from three straws of imported bovine semen belonging to the same batch as the one used on s 9(2, indicating the presence of *M. bovis* DNA, but culture attempts were unsuccessful. Although this does not provide any support for the presence of a viable organism in the semen, it leaves unresolved the issue of why this DNA could be identified in the semen. Further attempts could be undertaken to

isolate the organism from imported semen similar or identical to that used in the herds under investigation. If *M. bovis* is isolated, multilocus strain typing and whole genome sequencing can be used to clarify whether these isolates are closely related to the isolates recovered from s 9(2.

If s 9(2 is confirmed as the primary case, there is also the issue that the outbreak was explosive in nature, in the form of a point source epidemic that occurred in animals around the time of calving. This pattern of disease does not fit well with infected semen being used in the herd 9 months earlier. If further investigations identify s 9(2) as the primary case in this outbreak, the possible role of imported semen will need to be reassessed in the light of investigations that indicate that imported semen from the same source as s 9(2 was not used on this farm, contrary to initial indications.

s 6(c)

s 9(2)(ba)(i), s 6(c)

s 6(c), s 9(2)(ba)(i)

s 9(2)(ba)(i), s 6(c)

s 6(c)

s 6(c), s 9(2)(a)

s 6(c), s 9(2)(a)

s 9(2)(a), s 6(c)

Accidental human introduction

New Zealand dairy herds have a reliance on temporary workers from a variety of countries s 9(2)(a)(i). s 6(a)

However, fomites or biological materials illegally imported by migrant workers without malicious intent may be a source of introduction. Records of individuals employed on all possible primary case farms in the period preceding the outbreak should be collected to guide further consideration of this possibility.

[1] https://www.phe-culturecollections.org.uk/products/bacteria/detail.jsp?refId=NCTC+10131&collection=nctc

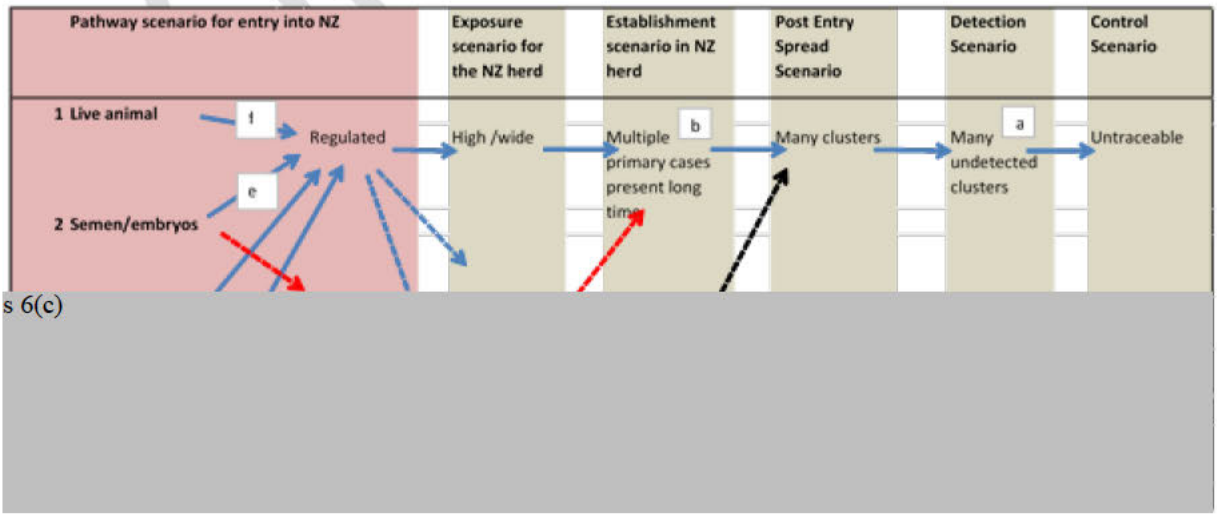
Live animal imports

Live cattle imports from countries other than Australia ceased in the late 1990s, and there have been no importations from Australia since 2013. Sanitary measures against *M. bovis* in imported cattle were introduced in 2006. There have been very few imports of live cattle, and the animals have gone into specialty herds, rather than the commercial dairy industry. MPI has also on two previous occasions undertaken surveillance to demonstrate that *M. bovis* was not at those dates endemic in New Zealand, and if infection had been introduced and spread sufficiently to be responsible for the current outbreak, endemic infection should have been detected by one of the previous surveillance activities, or at worst the current investigations should have shown up a very different distribution of infection from that found in the recent surveillance activities. As imported cattle are subject to registration and permanent identification under the Biosecurity (Imported Animals, Embryos, and Semen Information) Regulations 1999, it would however be a straightforward task to test these individuals, although it is considered a low priority activity.

The likelihood of introduction of *M. bovis* though any of the pathways described above is assessed to be very low, yet the event occurred, and unless some other new hypothesis can be identified, it seems that one of them was the mechanism in this case. There is no value in attempting to rank these from most likely to least likely, and all deserve investigation. To date MPI investigations appear to have been solely focussed on exploring the use of semen, with no attempt made to investigate the alternative pathways discussed either in the Entry Pathways Report or the additional possibilities described above. Whilst further work could be considered to investigate imported semen, the investigation of alternative pathways suggested above should be given higher priority.

A summary of the different entry pathways, and the expected epidemiological patterns that would flow from these entry pathways is shown in the following diagram (Figure 1).

Figure 1. Potential points of entry, transmission pathways and detection of *M. bovis* in the national dairy herd.



Pathways could be further investigated by

a. Bulk and discard milk surveillance nationally

b. Genotyping (MLST / WGS)all IPs; especially s 9(isolates

s 6(c)

9(2

s 6(c)

9(2)

9(

e. Attempting culture and genotyping of PCR positive semen batches

f. Testing imported bovine animals (not a priority).

Whether this outbreak is the result of a recent incursion of M bovis into New Zealand

The available evidence from the investigation to date supports a single recent introduction.

Previous surveillance for *M. bovis* provided provisional evidence that New Zealand was free of this organism, and the recent surveillance data presented to the TAG strongly indicates that *M. bovis* was not endemic in New Zealand prior to the current incursion. Although there remains a very low likelihood that *M. bovis* may have been present at low prevalence in cattle in New Zealand, in a small number of herds that were epidemiologically isolated from other herds, the weight of evidence supports the conclusion that the current outbreak is the result of a single point of introduction.

Whether a further incursion event is likely to occur again

The feasibility of successful eradication of *M. bovis* will depend upon the likely route of introduction (and therefore the likelihood of re-introduction). We have not identified a pathway that is associated with a high likelihood of precipitating another *M. bovis* incursion. As already noted, the likelihood of introduction of *M. bovis* though any of the pathways identified is very low and if further investigations indicate one of these pathways to be the route of introduction then further measures to reduce the likelihood of recurrence through that pathway should be considered. None of the evidence presented to the TAG has indicated that there is a previously undetected significant 'hole' in New Zealand's border biosecurity system, which would lead to further introductions of *M. bovis*.

Whether the index farm for this outbreak was the primary farm

Until the identification of s 9(2) , the evidence favoured the hypothesis that s 9(2 was the most likely site for the introduction of *M. bovis*, and therefore the primary case as well as the index case. In view of the very recent identification of infection on s 9(2) and the uncertainty about whether it became infected before or after s 9(2 , further investigation of s 9(2) is required to determine which of these premises (s 9(2)(b)(ii)) was infected first. Ongoing investigations could possibly determine that both s 9(2 and s 9(2) are secondary cases, and that there is at least one earlier farm in the infection chain.

TAG Task 3 Future options and the way forward

- ***Provide advice on the essential components of an eradication programme,***
- ***Provide an assessment of the likelihood of successful eradication using that programme,***
- ***Provide advice on how New Zealand could manage this disease if eradication is not attempted or is not successful,***
- ***Suggest suitable monitoring programmes for this disease if:***
    - ***Eradication is attempted, and***
    - ***Eradication is not attempted.***

Factors influencing the decision on control strategy

On the basis of the data presented to it, the TAG believe that eradication is technically feasible and is the preferred option. This is contingent on further surveillance confirming that infection is tightly clustered, and that there are no further farms infected that have no links to the existing two clusters (which may in fact be a single cluster).

The cost of a 'no control' option was estimated to be $NZ 800 million over 10 years by NZIER. While this analysis has limitations in its epidemiological and economic assumptions, it provides a useful starting point for policy development. The TAG considers that if it is to be revised or further developed, TAG members should be involved in ensuring that it makes use of the best available evidence.

A design limitation of this analysis is that it does not capture the potential negative effect on industry structure and animal value that may occur with endemic *M. bovis* infection. The NZ dairy industry has benefited from the sharemilking system, which enables relatively easy entry and progression for people within the industry. This system relies on the ability of individuals to acquire groups of cows that are mobile between farms over time. Were *M. bovis* to become endemic in some proportion of farms, this would result in a requirement to understand individual farm/cow group status to allow movement of stock. Herds or properties likely to be positive would be devalued and it is unlikely that herd owners would be willing to place stock on known infected properties and, conversely, positive herds would not be welcome on known likely free properties. This would substantially limit mobility within the industry, with negative economic effects. The economic and social impact of this may be substantial. The situation is further complicated by the substantial cost of testing herds to determine their status, and the remaining uncertainty after testing has been completed. Therefore, a herd accreditation program would be of limited value to the industry, unless more sensitive and specific herd tests could be developed.

Eradication

The TAG supports a phased process directed towards eradication of *M. bovis* from New Zealand.

The investigational activities since July 2017 have adequately demonstrated that infection is not ubiquitous, but because the mechanism of entry is still unknown, there may

be, as yet, unidentified local clusters of infection. These are most likely to be in the lower South Island, and the next step should be a complete bulk tank (and where possible pooled discard milk) testing of all herds in the lower half of the South Island by qPCR, or bulk tank ELISA testing as previously discussed.. This should be completed by late January. If this does not disclose any clusters unrelated to the current set of IPs, then it will provide confidence that the currently identified clusters are the only ones involved in the outbreak.

Organisms should be cultured from all current IPs and investigated by MLST and whole genome sequencing to determine whether one or more strains are present, with an initial focus on s 9(2) . If all isolates are closely and clonally related this will provide valuable evidence to justify eradication, and the degree of genomic variability found within the strain may provide useful hints about the approximate length of time since the incursion took place. If there is significant strain diversity between farms, this will indicate either multiple incursions or an earlier date of entry from a single incursion, suggesting that eradication may be less feasible.

Where trace forward procedures identify evidence of infection on the destination farm, as in the case of s 9(2 in Hastings, the first step should be to identify whether infection is limited to the introduced animals, or has spread to in-contacts. In the first case, all introduced animals should be promptly slaughtered and the remaining herd members should be tested using the standard protocol to determine whether the herd is infected or not. If in-contact animals are found through this surveillance to have become infected after initially being considered negative, then clearly transmission is still occurring, and the entire herd will have to be culled. If no in-contact animals are found through whole herd testing three times at intervals of at least three weeks plus qPCR testing of bulktank or waste milk as described earlier, then the herd can be considered negative. Any traces forward from herds that have received animals from IPs should be investigated to determine whether the infection chain has extended further and, if infection is identified, the herd should be investigated as for s 9(2 .

The goal should be to progress delimiting investigations and organism characterisation as far as possible by late February, and to use this evidence to formulate policy options based on epidemiological and economic criteria. If eradication remains the favoured option at that point, infected herds should be slaughtered at abattoirs as quickly as possible, and surveillance activities to achieve proof of freedom should be undertaken. The goal should be to complete the removal of infected herds by the end of April (when heifer rearing contracts end), and complete cleaning and disinfection by the end of May (gypsy day, when large numbers of animals move between farms). If any infected herds remain at that point, they should be subject to strict movement control.

Young stock moved from IPs may or may not be identified by trace forward. Known forward traces would become RPs and hence current containment will manage these. There is a risk of unrecorded movements, and hence assessment of this risk is required, by screening dairy animals born in 2016 and 2017 that could potentially have been exposed to infection. Evidence is that seroprevalence will be high in exposed calves. If we assume a test sensitivity of 0.75, an average calf mob size of 100 animals (i.e. 25% of a 400 cow herd) and

a likely seroprevalence of 10% or 20%, then 35 or 19 calves/group, respectively, need to be tested at least once by ELISA. The scale of this activity will need to be determined by an assessment of the risk based on epidemiological factors and the geographical location of potentially infected young stock.

It is noted that the value of slaughtered rising one- and rising two-year-old animals is less than that of mature animals. Thus, from an economic perspective, quarantining young stock and growing them out to more economic slaughter weights may be attractive. However the cost of maintaining effective biosecurity around these animals and potential ongoing liability for government for compensation of stock owners may be substantial. For this reason, the TAG's preferred position is that eradication proceed as fast as is reasonable within logistical constraints.

Zonal freedom

If the delimitation data suggest a prevalence of infection that indicates that national eradication would not be cost effective, a zonal freedom strategy may be feasible, as provided for under OIE procedures. Such an approach would involve eliminating any infection from a clearly defined geographical zone, and demonstrating that the zone is free of infection. The obvious initial candidate area is the North Island. The process could then be extended progressively to the north of the South Island, and potentially to areas further south.

This approach would allow current dairy farming practices to be maintained in the North Island, and herds could be moved from the North Island to the South Island, but not the other way, without being processed through a comprehensive herd accreditation program and quarantine procedures. It would require an officially managed program, using powers under the Biosecurity Act. The South Island could either be considered an endemic zone with no internal controls, or the industry there could operate a management program as described below.

Industry-managed control

An industry-managed approach could be implemented, with either no use of Biosecurity Act powers, or use of the Pest Management Strategy powers, in an industry-led program including Government participation. The first option would include industry managed education, testing and measures to reduce transmission between herds. It could potentially be managed via conditions of supply of milk to processing companies, since there would be no controls enforceable under statute. The second option could impose additional statutory control measures under the Biosecurity Act.

The exact details of an industry-led program would depend on the degree of spread of infection, the amount that industry would be willing to invest if there was no Government contribution, and the nature of the arrangement if it was a public-private partnership. The program could use the model of the bovine tuberculosis control program, which has been highly successful in reducing the level of TB infection in the national herd.

Experience in other countries has been that elimination of infection from a herd is not successful, and that the only way of re-establishing a disease-free herd is by culling all

animals and building a new herd. It may be possible to consider attempting other control strategies to clear herds of infection, but any approach would be exploratory, with a high probability of failure, judging by overseas experience.

Individual responsibility for protection from infection

M. bovis is endemic in many dairy industries around the world. In the absence of potential zoonotic significance and non-tariff trade barriers, it is feasible for individuals within the New Zealand dairy and beef industries to manage their herds to prevent entry of *M. bovis* to their herd and/or property, or to control the clinical expression of disease if they own an infected herd. As outlined above, this would, however, result in significant costs to the industry and would be technically challenging for individual owners to maintain. It would be likely to result in the disease becoming widespread throughout New Zealand. There would be some parallels with Johne's disease control, which continues to present challenges of a broadly similar nature.

Ongoing Surveillance

Surveillance should continue for 3 to 5 years before elimination of *M. bovis* is declared, because it is considered desirable to test bulk milk by qPCR when calves born in 2017 enter the milking herd and potentially circulate bacteria. This should cover all areas where cryptic infection may be present, using bulk milk qPCR as the screening method, with follow-up of all suspect herds.

Information and traceability systems

Successful identification of infected herds would benefit greatly from an effective animal traceability system. New Zealand has over the last few years implemented such a system, NAIT. This was the first time the system has been used for one of its primary purposes, and its value was limited by the failure of many farmers to fulfil their responsibilities under the system, making the tracing of animal movements far harder and less reliable than it should have been. Substantial improvement in compliance by herd owners, as well as making some changes in reporting procedures, will be required to ensure that NAIT fulfils its objectives more effectively.

The Ministry for Primary Industries has also invested heavily over at least a decade in information systems to support response management in the event of a disease incursion, but these also have performed poorly, and a plan is needed to provide better systems.

Deficiencies in information management have impacted severely on the speed and operational efficiency of the incursion response.

TAG Task 4 Disease presentation and impacts

- ***Through a combination of experience of working with this disease overseas and knowledge of NZ-specific farming practices, provide advice about:***
    - ***Whether the disease is presenting differently in NZ,***

- ***The factors that are most likely to be contributing to the disease presentation/s being seen, and***
- ***The likely impacts on farming practices should this disease establish in New Zealand.***

Whether the disease is presenting differently in NZ, and the factors that are most likely to be contributing to the disease presentation/s being seen

The s 9(2)(b)(ii) herd may be atypical with respect to the neonatal calf disease, the abruptness and extent of disease in the dry cows, premature birth of calves and the peculiar presentation of front-fetlock single-joint disease. We consider the neonatal calf disease likely to have resulted from septicaemia, resulting in the lesions of arthritis, peri/epicarditis, meningitis and interstitial pneumonia. These findings are consistent with a high-dose haematogenous infection, such as *in utero* infection with *M. bovis*, probably associated with placentitis, which could cause the premature births. With respect to disease in the milking cows, single-joint infection can occur, although polyarthritis is more typical; single-joint infection as a consistent finding in multiple animals, as in s 9(2), has been reported by others. A caveat is that we do not have verifiable clinical evidence or any *post mortem* evidence that other joints were not infected.

Mastitis in dry cows was the prominent feature of disease at s 9(2), and an apparent origin of an outbreak in dry cows has been reported previously. Although the lesions of *M. bovis* mastitis are poorly described in the literature, the lesions in s 9(2) dry cows are consistent with *M. bovis* mastitis. Several TAG members considered it unusual that the July-calving cohort of dry cows had such a high (200/380, >50%) prevalence of mastitis that uniformly affected all 4 quarters; one TAG member considered this not inconsistent with other outbreaks. Such occurrence of multiple-quarter infections is not unusual in severe mycoplasma mastitis outbreaks, and is consistent with either an intramammary or a haematogenous route of infection of the mammary gland. An unusual feature is the dichotomy between unaffected cows and cows with disease in all 4 mammary quarters, but apparently no diseased dry cows with involvement of fewer mammary quarters. It may seem striking that as many as 200 dry cows were affected, but this represents 50-60% of the animals at risk and on a percentage basis is not beyond what has been reported by others in documented case studies of mycoplasma mastitis outbreaks. Nonetheless, the unusual features of the disease presentation in this herd suggest an atypical method of introduction of infection, as discussed elsewhere in this report.

The situation may be similar in herd s 9(2)(b)(ii) The lung lesions in calves from this herd were typical of infection with *M. bovis*. Kidney lesions were identified in calves from s 9(2), but it is not yet convincing that they were a direct result of *M. bovis* infection; as already noted, there appears to have been little investigation of other concurrent endemic or exotic agents.

Other herds presented in a conventional manner with a lower frequency of infection and mastitis s 9(2)(b)(ii) and other herds with no clinical disease (including s 9(2)(b)(ii) and s 9(2)(b)(ii)). These observations of secondarily infected herds do not suggest a strain that is hypervirulent or that results in unique disease manifestations. Reports of outbreaks of *M. bovis*-associated bovine diseases occurring in clusters of herds were not unusual in the western USA in the 1970s.

The likely impacts on farming practices should this disease establish in New Zealand.

Impact on dairy herds

M. bovis, in general, is known to be highly contagious within herds, based on experience in other countries, but spread between herds can be prevented by movement restrictions and related measures. The major risk factor for transmission between herds is an infected animal that is excreting organisms, quite possibly intermittently. The organism is not known to transmit by mechanisms such as windborne spread between herds, which could bypass movement control. The specific strain present in New Zealand is behaving much as in overseas experience, as evidenced by:

(a) its apparent entry into herd s 9(2)(b)(ii) with only 3 days of exposure (17-July),

(b) its entry into herd s 9(2)(b)(ii) despite no calf-to-calf contact, with an apparent breakdown of the producer's quarantine attempt, and

(c) evidence of spread to herds s 9(2)(b)(ii) outside of the s 9(2) business structure.

It is notable that animal movement into and out of the s 9(2) business structure was limited, whereas the New Zealand dairy industry in general is characterized by much more frequent movement between enterprises. Thus, it is likely that *M. bovis* would spread widely in New Zealand dairy herds if it is not contained. **Furthermore, large herd sizes and extensive animal movements (characteristic of the NZ dairy industry) are factors associated with a high prevalence of *M. bovis* infection and disease in other countries**. We expect the principal manifestations to be chronic non-responsive mastitis and arthritis, as has already been seen, and respiratory disease as the infection becomes established in young stock, through the feeding of infected milk. In initial outbreaks as many as 50%-60% of animals may be affected, as seen in s 9(2. Affected animals are often culled due to arthritis and pneumonia, as well as mastitis. Milk somatic cell counts can exceed 1,000,000 cells/ml in infected cows and could raise the bulk tank milk somatic cell count excessively.

Impact on beef herds

It seems likely that *M. bovis* would enter into beef herds (if infection in dairy herds were not contained), because of the contagious nature of *M. bovis* in general and the New Zealand strain specifically, and the interactions between the beef and dairy cattle populations through dry cow overwintering and the rearing of dairy calves for beef, with an estimated 0.48 m calves transferred from dairy to beef farms. The disease impact of the *M. bovis* incursion on the beef population is less clear. On one hand, the low animal density on pasture is expected to reduce the disease risk. On the other hand, the moderate herd sizes (320 beef animals per farm) and the apparent recent trend to use of makeshift feedlots are factors that increase the disease risk. A key to estimating the risk would be knowledge of the amount of co-mingling of animals from different herds, as this seems to be a key risk factor. Respiratory disease and polyarthritis are the expected manifestations of *M. bovis* infection in beef cattle; current IPs have not experienced significant respiratory disease, but we expect that this is a consequence of the types of animals infected and perhaps the route of infection, and not a characteristic of

this *M. bovis* strain. Indeed, calves at s 9(2)(b)(ii) had lung lesions characteristic of *M. bovis* infection, and it is well-recognized that the same strain is capable of causing mastitis, pneumonia and/or arthritis, depending on the situation. Finally, the co-mingling and confinement of dairy calves raised for beef imply that *M. bovis* would cause respiratory disease and arthritis in this population. Thus, we expect there could be an impact on the NZ beef industry, but the magnitude of the impact depends on the above factors.

Significance of NZ's naïve cattle national population

For most infectious agents, an incursion is expected to cause an initially high incidence of severe infection in the initially naïve population, with a later reduction in incidence of both infection and disease as the disease becomes endemic, and population immunity increases. *M. bovis* is unusual because it is not clear that recovered animals are necessarily resistant to infection, and in fact many *M. bovis* infections are chronic. Thus, it should not be assumed that disease would become less frequent as *M. bovis* infection becomes endemic in the national cattle population.

TAG Task 5 Disease control operations

- ***Provide an evaluation of the suitability of the disease control protocols that have been used in this response, including:***
  - ***Controls for Infected Places,***
  - ***Controls for at-risk places under movement control notices,***
  - ***Controls for farms that have been depopulated.***

From the data provided, it appears that the disease control protocols are adequate.

Controls for Infected Places

These have been implemented effectively. It is important that when entire herds are slaughtered that compensation is calculated in a transparent manner and paid promptly, so that the owners of animals and farms (who are severely affected both financially and emotionally) are fairly treated, so that other people remain willing to provide information and comply with all restrictions that may be imposed on them.

Controls for at-risk places under movement control notices

This is a challenging group, particularly in this incursion response, where tracing activities are complex and time-consuming, and farms that come under a cloud can be severely affected, especially if their status is not resolved for some time. With the requirement for three negative tests before a Restricted Place is declared negative, resolution can be slow. The interval of at least three weeks between tests is substantial, but appears necessary, in order to detect newly introduced infection.

Controls for farms that have been depopulated

Farms should be cleaned and disinfected following depopulation, and then remain destocked for 60 days after completion of cleaning and disinfection. Wilson *et al.* (2011) reported that *M. bovis* may survive in sand for 11 weeks. However, there was no evidence that cow

transmission occurred, as the cow and sand isolates were genetically distinct. Thus the 60 day destocking period is defensible.

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