Ministry for Primary Industries
Manatū Ahu Matua

Analysis of risk pathways for the introduction of *Mycoplasma bovis* into New Zealand

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Manatū Ahu Matua

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Executive summary

Mycoplasma bovis was identified in a dairy herd in the South Island of New Zealand on 22 July 2017. This was the first report of the organism in New Zealand and a response under the Biosecurity Act was initiated as *M. bovis* was considered an exotic risk organism (Unwanted Organism).

Surveillance activities carried out prior to and including the response have provided no evidence that *M. bovis* was endemic to New Zealand dairy cattle. This included a serological survey in 1995 (Reichel *et al.* 1999) and a national bulk milk surveillance study in 2007 (McDonald *et al.* 2009). Further to this, routine exotic disease investigations carried out continuously as part of the New Zealand's passive surveillance system have not detected the organism. An additional portfolio of surveillance activities has been carried out as part of the ongoing *M. bovis* biosecurity response and has to date provided no evidence that *M. bovis* was present in New Zealand beyond known infection networks from the index farm detected as part of the response s 9(2)(a) 2017). Studies completed on infection timelines also point to the outbreak index property being the primary infected farm.

Data accumulated as part of the response to this point do not support silent and very low-level endemic infection; however, it remains an alternative explanation for the disease cases observed on the affected enterprise. The base assumption of this analysis is that *M. bovis* was exotic prior to the recent detection and that the infection pathway into the affected enterprise is likely to have originated from an overseas rather than from an endemic (but yet an undetected) local source. Further surveillance work will continue to be undertaken to affirm the validity of this assumption. The output of this report reflects our best understanding at the time of finalisation of this report; however, this response is still active and should further evidence become available we recommend an update of this analysis that considers the endemic scenario and/or re-assesses the relative importance of the other pathways.

Using available information and a combination of assessment methodologies the following conclusions were made by the working group:

- There are seven potential introduction pathways i.e. imported live cattle, imported frozen semen, imported embryos, s 6(c) imported feed, imported used farm equipment and other imported live animals.
- The group expert assessment concluded that, while none of the potential pathways should be excluded, some pathways were more likely to have caused the outbreak (i.e. imported frozen germplasm) than others (imported live cattle, imported used equipment).
- The seemingly one-off nature of the outbreak might be explained by a failure of existing border measures to prevent entry or entry through an unregulated/illegal pathway, the detail of which is currently unknown.
- An Australian infection source is believed to be unlikely on the available evidence. This perspective is based on there being no recent live cattle imports

from Australia and the evidence provided by genomic analysis of the New Zealand *M. bovis* strain.

- Imported frozen semen has been widely speculated as a presumed pathway for the introduction event into the affected enterprise. However, our analysis has revealed some considerable weaknesses around this theory. These include: hundreds of thousands of semen straws from endemic countries have entered New Zealand without any evidence of previous incursions, absence of studies showing transmission of the disease via semen, and no significant changes in risk management strategy for nearly three decades. Therefore, it is critical that alternative explanations continue to be explored.
- This analysis has further highlighted the critical importance of baseline surveillance data to benchmark the presence or absence of pathogens in New Zealand and to support disease response activities such as the *M. bovis* 2017 Response.

The following recommendations were made by the group:

- Given the knowledge gaps regarding the infection risk from germplasm (i.e. frozen semen and embryos), research funding needs to be allocated to better understand the transmission risk posed by these imported commodities. Thus it is recommended that a research proposal is developed to explore, in particular, frozen semen as a conveyer of *M. bovis*. This research could include presence and infectivity of *M. bovis* in semen following experimental infection of bulls. The output of this research may be used to inform on the need for and the expected effectiveness of additional risk mitigation steps.
- Locate imported cattle and work up a survey on the status for *M. bovis* in these animals if it is technically feasible (i.e. accounting for things such as availability of an appropriate negative control, duration of *M. bovis* antibodies in relation to import date of cattle etc.).
- Continue to suspend live cattle imports until further risk assessment work has been completed.

Introduction

In response to the July 2017 detection of *M. bovis* a multi-disciplinary working group consisting of participants from government and industry with support from an independent consultancy were tasked with assessing the relative likelihood of known introduction pathways for *M. bovis* into New Zealand and into the affected enterprise.

For completeness and to ensure that all known risk pathways were addressed in sufficient detail, between August and November 2017, a series of studies and assessments were conducted by the expert working group. While data on all pathways was collated, several knowledge gaps were identified (for instance lack of studies confirming transmission of *M. bovis* via frozen semen) that hindered the group's ability to understand the risk posed by the various conveyers of *M. bovis* related to these pathways. However, the group used their scientific expert judgement to generate a high-level relative assessment of the risk pathways

The objectives of the work were defined as follows:

1. To identify and describe risk pathways for the introduction of *M. bovis* into New Zealand prior to July 2017.
2. To produce a high-level relative assessment of the identified pathways building on both existing scientific evidence as well as outputs from the ongoing *M. bovis* response.

To achieve the objectives identified a series of face-to-face expert meetings were conducted to exchange data and expert opinion and to define the methodology for the assessment. Subsequent to these initial consultations the following key import risk pathways were identified by the group (Figure 1):

- Bovine *in vivo* embryos
- Bovine semen
- Bovine feed
- Used equipment
- Live animals (non-cattle)
- Live cattle (from Australia)
- s 6(c)

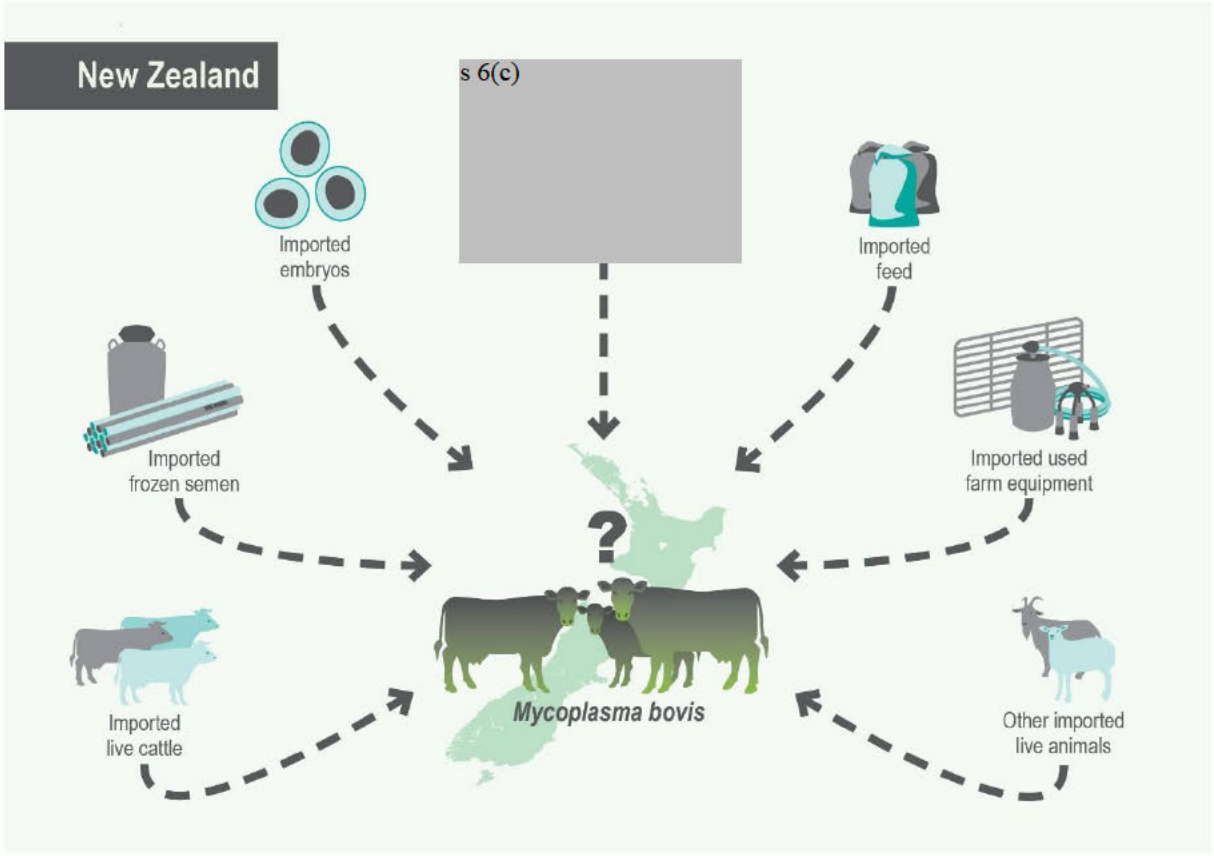

Figure 1: Identified key pathways for introduction of *Mycoplasma bovis* into New Zealand.

Different MPI risk documents were compiled and updated to support the overall analysis; namely:

- Rapid risk assessment semen (MPI, 2017a)
- Rapid risk assessment *in vivo* embryos (MPI, 2017b)
- Rapid risk assessment live animals (non-cattle) (MPI, 2017c)
- Rapid risk profile bovine feeds, used equipment, s 6(c) (MPI, 2017d)
- Import risk analysis for live cattle (MPI, 2009)

Further the assessment was informed by the following information and data sources:

- National surveillance data carried out as part of the biosecurity response to delimit infection and understand the status of New Zealand prior to the incursion;
- Molecular subtyping and whole genomic sequencing of the New Zealand strain of *M. bovis*;
- Analysis of import data on potential conveyers of *M. bovis*;
- Analysis of semen used on the affected enterprise.

Building on these individual pieces of evidence, the working group applied a Multi-Criteria Risk Assessment (MCRA) methodology to create a first high-level relative assessment of likely risk posed by the different pathways. A group of criteria were

identified which contribute to the potential of each of the investigated pathways to have caused the 2017 incursion.

The following criteria were considered:

- Probability of presence of *M. bovis* in imported commodity
- Number of importations per year
- Likely viability of *M. bovis* in imported commodity
- Epidemiological plausibility of pathway
- Frequency of contact with imported commodity

For each criteria the pathways were ranked through consensus opinion of the involved experts. Based on the results for each pathway an overall risk level was assigned to each pathway. To ensure the best possible process under the given constraints of both time and scant available scientific literature, the assessment was peer-reviewed by several independent reviewers.

Summary of available evidence

MPI RISK DOCUMENTS

The risk documents (rapid risk assessments for bovine semen, bovine embryos and live animals, rapid risk profile for bovine feed, used equipment as well as s 6(c)) and other analyses (Import risk assessment of live cattle, import data, interim surveillance results and dedicated studies) form the basis for this risk pathway report. These documents, currently in the process of internal and external peer-review, were produced for the purposes of informing the pathway analysis presented in this report. They will become available as standalone documents once peer review is complete. High-level preliminary summaries of their outputs are presented below.

Bovine semen (MPI, 2017a)

The isolation of viable *M. bovis* in the semen of clinically normal bulls has been demonstrated infrequently (Jain *et al.* 2012; Trichard and Jacobsz 1985; Stipkovits *et al.* 1983; Jurmanova and Sterbova 1977; Langford 1975). However, once present in semen *M. bovis* can survive for prolonged periods and does not appear to be eliminated by processing or freezing (Hirth 1967). It has also been demonstrated in two independent studies that the antibiotics commonly used in semen extenders may not be completely effective in eliminating *M. bovis* in all cases (Shin *et al.* 1988; Visser *et al.* 1998).

However, there is no documentation of natural transmission from semen collected and processed under the internationally accepted protocols. There is limited experimental evidence demonstrating pathogenicity of *M. bovis* on the reproductive tract (Hartman *et al.* 1964; Hirth 1966). There is a plausible but unproven method of *M. bovis* transmission via infected semen. There has been inadequate study of this particular pathway, partly attributable to the fact that so few countries are free from the disease

and have few significant drivers to understand the risks, and consequently there is a paucity of scientific data.

On the basis of currently available scientific evidence the likelihood of viable *M. bovis* being present in semen is assessed to be low, but non-negligible[1]. Given that imported semen is inseminated into susceptible animals the likelihood of exposure is certain. However, based on what is currently known the likelihood of *M. bovis* transmitting to an exposed recipient is highly uncertain. Therefore the likelihood of transmission is assessed to be very low, but non-negligible. The consequence assessment for the entry and establishment of *M. bovis* is moderate. The risk estimate is therefore non-negligible and *M. bovis* is considered a risk in imported semen.

Measures to manage the risk of *M. bovis* in bovine semen have been set by the relevant import health standards.

Bovine embryos (MPI, 2017b)

M. bovis has been identified infrequently in the reproductive tract of both clinically normal cows (Jain e*t al.* 2012; Langford 1975) and in cows demonstrating reproductive disorders (Stipkovits 1996). The presence of *M. bovis* in the reproductive tract provides the potential for contamination of embryos or oocytes collected from infected donors. Experimental studies have demonstrated that both *in-vivo* and *in-vitro* produced embryos may retain *M. bovis* infectivity despite standard processing procedures including washing of embryos in accordance with International Embryo Technology Society (IETS) protocols, trypsin treatment and exposure to antibiotic combinations (Bielanski *et al.* 2000 ; Riddell *et al.* 1989; Bielanski *et al.* 1989; Riddell *et al.* 1993a ; Riddell *et al.*1993b).

The likelihood of transmission of *Mycoplasma* species associated with *in-vivo* produced embryos has been categorised by the IETS as Category 4, meaning "*studies have been done, or are in progress, that indicate that no conclusions are yet possible with regard to the level of transmission risk; or the risk of transmission via embryo transfer might not be negligible even if the embryos are properly handled in accordance with the IETS Manual between collection and transfer." (OIE 2017)*. Similarly with *in-vitro* produced embryos there is uncertainty relating to the likelihood of transmission as a result of inadequate study of this particular pathway and a paucity of scientific data.

Thus the likelihood of entry is assessed to be low, but non-negligible. The likelihood of exposure is certain since imported embryos are directly implanted into the recipient animals. However, significant uncertainty relates to the likelihood of transmission should *M. bovis* be present in embryos. On the basis of currently available scientific evidence, and considering the categorisation of *Mycoplasmas* by the IETS, the likelihood of transmission is assessed to be very low, but non-negligible.

[1] Within the Import Risk Analysis framework the likelihood of entry and exposure are considered as being either "negligible" (not worth considering; insignificant) or "non-negligible" (worth considering; significant). Where possible descriptors such as "very low" (close to insignificant), low (less than average), medium (average level), high (extending above the average level), very high (well above the average level) are used to describe the comparative levels of non-negligible likelihood. Whilst these descriptors are qualitative and potentially subject to linguistic uncertainty, calibration of the estimate is performed through both the internal peer review process and external review process.

In New Zealand the importation of bovine embryos is limited to *in-vivo* produced embryos. Measures to manage the risk of *M. bovis* in bovine *in-vivo* produced embryos have been set by the relevant import health standards.

Bovine feed, used equipment and s 6(c) **(MPI, 2017c)**

s 6(c)

Live cattle and other imported animals (MPI, 2009 and 2017c)

Live cattle

Cattle can be subclinically infected with *M. bovis* and can continue to shed the agent over extended periods of time (Nicholas *et al.* 2016). The likelihood of entry of *M. bovis* in live cattle is therefore assessed to be non-negligible. Since imported cattle are integrated into New Zealand cattle herds the likelihood of exposure of New Zealand cattle to *M. bovis* infected imported cattle is high. The consequence assessment for the entry and establishment of *M. bovis* is moderate. The risk estimate is therefore non-negligible and *M. bovis* is considered a risk in imported live cattle.

Measures to mitigate the risk of *M. bovis* in cattle imported from Australia were provided by the relevant import health standard. These measures have been implemented from 2006 to the time of suspension of the standard in August 2017. However, no live cattle have been imported since 2013. Prior to the adoption of import health measures in 2006, opportunity for entry of this organism into New Zealand existed via the importation of live cattle. Cattle from countries other than Australia have not been imported since the late 90s.

Other imported animals

Although most species of *Mycoplasma* are very host specific, there are infrequent reports of *Mycoplasmas* in hosts other than their perceived natural host animal species. Non-bovine species susceptible to infection with *M. bovis* include sheep, goats, deer and broiler chickens and pigs (Kumar *et al.* 2012; Bocklisch *et al.* 1987; Ayling *et al.* 2004; Egwu *et al.* 2001; Dyer *et al.* 2004; Ongor *et al.* 2008; Spergser *et al.* 2013). Of these species, only those currently imported into New Zealand were considered as part of this rapid risk assessment i.e. sheep, goats and deer.

There is just one published report of *M. bovis* in deer from North America (Dyer *et al.* 2004). Given the lack of evidence for frequent occurrence of *M. bovis* in deer, the likelihood of it being associated with imported deer from Australia is concluded to be negligible.

Reports of natural transmission of *M. bovis* to sheep and goats with resultant disease are equally rare. The agent has been isolated from a mastitic goat in the UK (Ayling *et al.* 2004), and from mastitic goats in Nigeria (Egwu *et al.* 2001). Further, a study by Kumar *et al.* (2012) reported an outbreak of pneumonia in sheep where *M. bovis* was isolated from tracheal and lung samples. A study by Bocklisch *et al.* (1987) examined the pathomorphologically altered lungs of 233 sheep for the presence of *Mycoplasma* species and reported the detection of *M. arginine*, *M. bovis* and *A. laidlawii*. However, the transmission of *M. bovis* from sheep and goats to cattle has not been demonstrated. Thus the likelihood of entry of *M. bovis* with live sheep and goats from Australia is assessed to be very low but non-negligible. The likelihood of exposure is negligible and therefore the risk estimate is negligible and *M. bovis* is not considered a risk in other imported live animals.

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

OTHER INFORMATION SOURCES

Analysis of semen used on the affected enterprise

Imported semen used by the affected enterprise leading up to the outbreak was tested in a pilot study. *M. bovis* DNA was detected using PCR and DNA sequencing in approximately 6% (3/54) of the semen straws. However, these results must be interpreted very cautiously as *M. bovis* was not able to be cultured from PCR-positive straws. Thus the results do not imply that any viable *M. bovis* was present in the semen samples and only confirm the presence of DNA. An effort was made to type the samples directly from semen using a technique called Multi-locus Sequence Type (MLST). Unfortunately the *M. bovis* DNA quantity was not sufficient to give a

result. As part of the pilot studies additional batches of semen were tested from the above bulls with PCR positive results, and no further *M. bovis* DNA was detected.

Therefore in the absence of further evidence the only valid interpretation is that contamination of imported semen with *M. bovis* DNA is not an uncommon finding. Further, since the sample size in this first study was very small, more work would be needed to confirm the results and to make inferences about the overall level of *M. bovis* contamination likely in imported semen.

In conclusion, this pilot study does not provide conclusive evidence that *M. bovis* was transmitted to cows at the affected enterprise by imported semen. The plausibility of this pathway rests on it being an extremely low probability event (given the absence of previous detections despite the large number of import events over 40+ years), and the pathway can neither be confirmed nor excluded at this point.

Phylogenetic analysis

The genomes of 16 New Zealand (NZ) *M. bovis* isolates sequenced at MPI confirmed that all isolates were *M. bovis*. Analysis was undertaken to compare the New Zealand *M. bovis* isolates with isolates of *M. bovis* from other countries using the genomic data and a technique called Multi-locus Sequence Type (MLST). These analyses are dependent on making comparisons to available sequences in the international datasets. Unfortunately these datasets are limited and not a fully representative picture of the globally spread *M. bovis*. The New Zealand isolates were all found to be the same sequence type (ST) by MLST genotyping which is unreported in the available international databases. This was independently confirmed by the *Mycoplasma* Reference Laboratory at the Animal and Plant Health Agency, Weybridge, UK based on testing a single New Zealand isolate. The MLST analysis indicates that the isolates detected in New Zealand are more closely related to some isolates reported from Europe, Israel, the United States of America and Japan, rather than the strains reported in Australia (Figure 2). So far comparisons of the New Zealand isolates using whole genome analysis are consistent with the MLST results and in the absence of data from Europe, generally show a closer relationship to the USA genomes than China or Australia. Due to the limited genomic information on *M. bovis* in the international databases and the lack of exact matches of the New Zealand strain, at this time the probable origin of the New Zealand isolates cannot be robustly inferred.

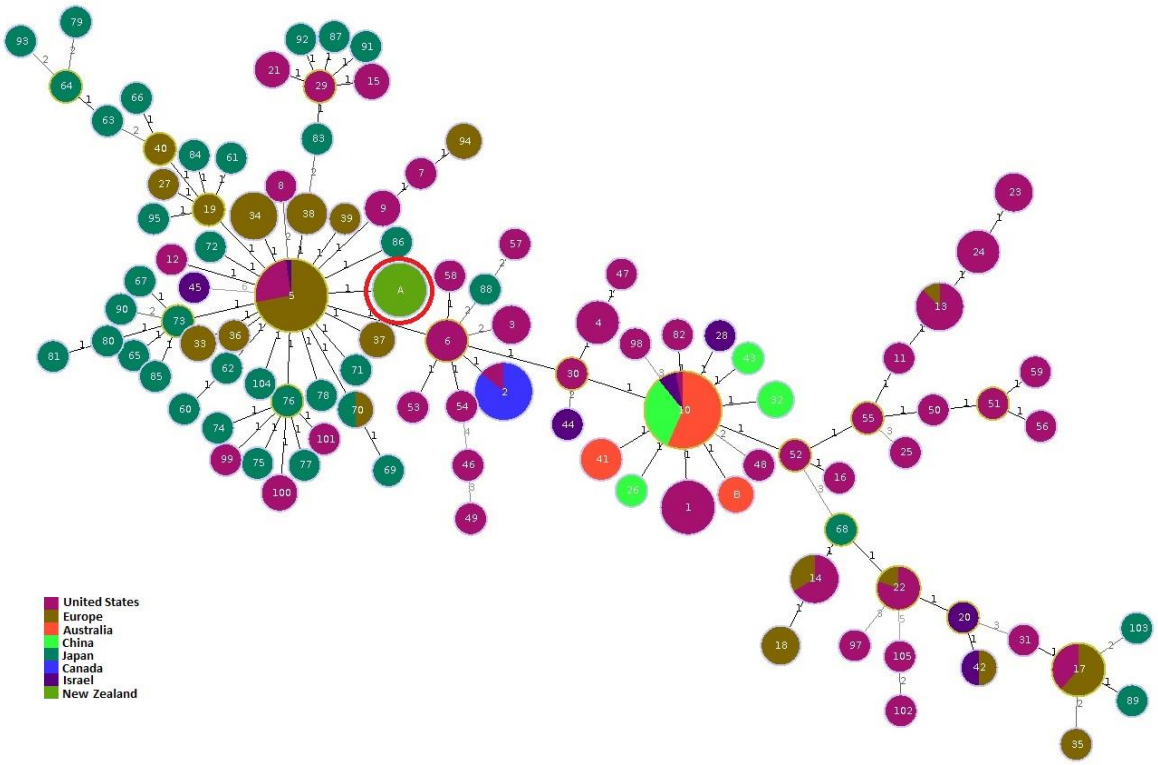

Figure 2. Minimum spanning tree (MST) showing genetic relationships of New Zealand isolates of *Mycoplasma bovis* with isolates from different geographical locations based on the pubMLST genotyping system. Each geographical origin of the isolate is represented by different colour and each circle represents a unique sequence type (ST). The size of the circle corresponds to the number of isolates and the numbers on the connecting branches represent the number of locus variation between each connected ST i.e. 1 is a single locus variant (SLV) and 2 is a double locus variant (DLV). New Zealand isolates were highlighted by red circle. This analysis is based on the pubMLST profile created using the global optimal eBURST (goeBURST) algorithm in the PHYLOViZ software. The analysis was performed on pubMLST data updated on 10 November 2017.

Analysis of imported conveyers (of *Mycoplasma bovis*)

Available information on imported conveyers was compiled (Figure 3). Over the past ten years there have been 110 imports of bovine species (from Australia only), 18 to the South Island and 92 to the North Island. No imports of bovine species (cattle and buffalo) have been recorded since 2013. Thus, whilst live animals might be considered to be the most efficient and likely pathway for introduction of *M. bovis* it would seem that any introduction from this pathway would need to have been historic given the lack of recent imports. Cattle germplasm imports into NZ started in the 1950s. An annual average of 566 embryos were imported over the last ten years, with total number imported for this period of 6225. For semen the average number of straws imported per year and the total for the period were 230,000 and 2,518,172, respectively.

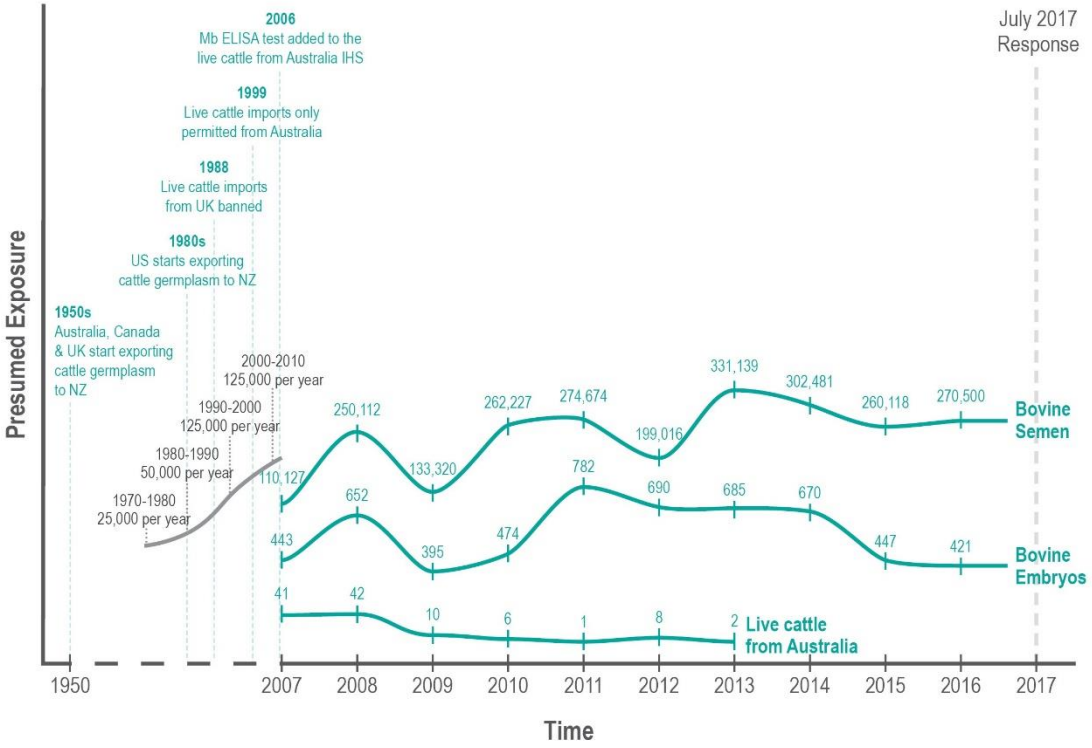

Figure 3: Information on historic potential import events of *Mycoplasma bovis into New Zealand.*

Surveillance

Surveillance activities carried out prior to the response have provided no evidence that *Mycoplasma bovis* was endemic in New Zealand dairy cattle. This included a serological survey in 1995 (Reichel *et al.* 1999) and a national bulk milk surveillance study in 2007 (McDonald *et al.* 2009). The 1995 and 2007 surveys were designed to detect the organism at 2% and 3% prevalence (i.e. the minimum expected prevalence if disease were present). Hence they provided evidence that at the time of the surveys the disease was not present at a level equal to or greater than 2 and 3%, respectively. Further to this, routine exotic disease investigations carried out continuously as part of the New Zealand's passive surveillance system have not detected the organism. An additional portfolio of surveillance activities has been carried out as part of the current *M. bovis* biosecurity response and has to date provided no evidence that *M. bovis* was present in New Zealand beyond known infection networks from the index farm detected as part of the response s 9(2)(a) 2017). Studies completed on infection timelines also point to the index infected property (within the affected enterprise) in the outbreak being the primary case farm of the current outbreak.

Further, data accumulated as part of the response to this point do not support silent and very low-level endemic infection throughout New Zealand; however, it remains an alternative explanation for the disease cases observed on the affected enterprise.

Surveillance conducted as part of the *M. bovis* response included two main streams of activity; i.e. 'response surveillance' and 'national surveillance'. The purpose of response surveillance is to collect information on farms associated with the affected

enterprise (therefore it consists of enterprise farm surveillance, trace farm surveillance i.e. surveillance of those farms connected by movement of cattle between properties, and surveillance of contiguous property farms). Trace surveillance has also included investigation of associated properties that share similar risk profiles to the affected enterprise. In this context trace surveillance has included PCR testing of bulk milk from dairy farms that have been exposed to the same imported semen batches as the affected enterprise.

In contrast, national surveillance is conducted for the purpose of determining the presence of *M. bovis* outside of the affected enterprise and associated network. To date national surveillance has included the following activities:

- Investigation and testing of suspect cases (that could fit with the clinical case definition of *M. bovis*) reported by private veterinarians (often referred to as report case surveillance);
- Active surveillance (census testing) of bulk milk and discarded milk, i.e. milk s 6(c) from dairy farms in the two districts surrounding the affected enterprise;
- Risk-based surveillance of cattle farms across New Zealand. Practicing large animal vets were contacted and requested to identify farms which met a case definition suggestive of *M. bovis* infection. Various samples were collected and a brief questionnaire was carried out on these farms. This activity aimed to cover cattle herds across the whole of New Zealand;
- Enhanced passive surveillance through census testing by PCR of all mastitic milk submitted to the regional laboratories;
- Active surveillance of beef feedlots through testing (serological and PCR) of livestock at slaughter plants.

Most, if not all of these response surveillance activities can be considered risk-based and of greater sensitivity than general surveillance activities such as routine passive surveillance. Further surveillance work is currently being undertaken. One anticipated outcome will be using the outputs from the array of surveillance activities completed as part of the response to calculate the overall confidence of disease freedom (outside of the known infection network) at a defined intra and inter-herd prevalence.

Interview with key decision makers of the affected enterprise

An interview was conducted with the key decision makers for the affected enterprise. The interview was focused on identifying introduction of conveyers of *M. bovis* associated with key import risk pathways. There was no indication during this interview of any specific import event that could have explained the introduction of *M. bovis* into the enterprise.

Pathway analysis

The overall expert opinion-based assessment for the assessed pathways is summarised in Figure 4. The analysis qualitatively describes the relative importance of each pathway. For each criteria the pathways were ranked through consensus

expert opinion of included experts (n=6). Based on the results for each pathway an overall probability level was assigned to each pathway.

Importantly for all pathways the risk has been assessed as low, if not negligible or very low. Thus it is important to note that even if considered more likely, none of the pathways investigated should be considered a high-risk pathway for the introduction of *M. bovis* into New Zealand.

Figure 4: Multi-criteria risk pathway matrix illustrating the relative importance of group of identified key pathways for the 2017 *Mycoplasma bovis* detection. Note, null was used to describe the number of cattle importations as no imports had occurred in the recent past. Generally each criteria is described on a 4-level scale from light (=lowest) to dark (=highest) orange. Importantly, for all pathways the risk has been assessed as low, if not negligible or very low.

Following the matrix assessment and looking specifically at what is currently known about the affected enterprise. Figure 5 provides an update on the role of the individual pathways.

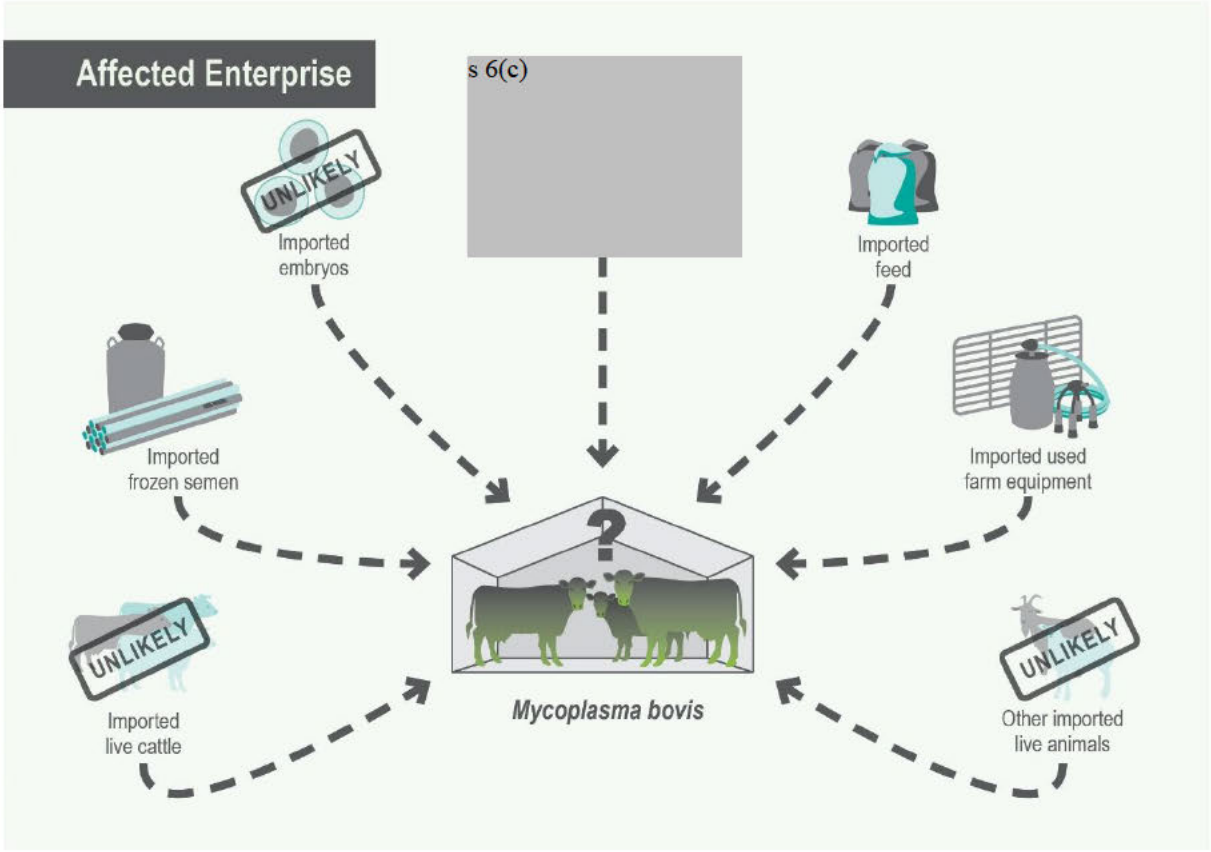

Figure 5: Identified key pathways for introduction of *Mycoplasma bovis* into the affected enterprise 2017.

Conclusions and recommendations

This report aims to provide a high-level relative assessment of the import risk pathways that could have led to the July 2017 outbreak. Our analysis makes the base assumption that the outbreak has occurred as a result of a recent incursion and doesn't consider the possibility of endemic spillover. Analysis is currently underway to define the confidence that New Zealand was free of *M. bovis* at a design prevalence of a 1% herd infection. The aim of the analysis is not to conclude the exact cause of the 2017 *M. bovis* outbreak, but rather to provide a multidisciplinary expert perspective on the relative likelihood of different pathways being responsible for *M. bovis* entering the country and the affected enterprise. Notably, no high-risk pathway for introduction of *M. bovis* has been identified, but rather a group of pathways posing a continuous but non-negligible, very low to low risk.

Import of live cattle from Australia might have been considered to be the most likely pathway for entry of *M. bovis*, given the known efficiency of animal-to-animal contact in the transmission of the disease and the known rate of infection with the disease in

Australia. However, the absence of recent cattle imports along with the genetic analysis suggests that this pathway is less plausible than others.

Despite live cattle imports occurring over many decades in the past with significant risk of carrying *M. bovis*, it is plausible that rare introductions into New Zealand e.g. through live cattle imports or potentially through germplasm may have occurred yet failed to establish endemic (or epidemic) infection in the country. Localised fading out of *M. bovis* infection within herds in endemic countries appears to be not uncommon (e.g. Punyapornwithaya *et al.* 2012). Under more extensive, open air pastoral farming conditions in New Zealand (particularly in beef), within-herd transmission is generally expected to be less efficient, thus increasing the likelihood of extinction of infection (without spread or clinical outbreaks); that said poor biosecurity in these systems would not necessarily prevent between-farm spread. Thus it is conceivable that failure to detect established infection in New Zealand (by intensive surveillance as well as targeted investigations) and possible absence of endemicity is compatible with a high risk pathway (live cattle imports) in the past.

Whilst the approach we use provides a high-level perspective on the relative risk of pathways assessed it cannot confirm how *M. bovis* entered into the specific affected enterprise and thus into New Zealand. One of the reasons for this is that current evidence suggests that only a single outbreak has been observed. Thus there is no evidence for repeated wide-spread exposure of our cattle population to the disease agent.

Although this assessment has highlighted the risk posed by semen, it is important to consider the likely level of risk presented by this commodity assuming it was the cause of the incursion. The epidemiological picture that is currently being observed of *M. bovis* in New Zealand is that of a single incursion resulting in an outbreak. The median number of semen straws imported annually over the last ten years was 250,000 thus equating to a risk of approximately 1 in 2.5 million for that period. If all imports since the 1970s are considered the risk posed by a single straw may be estimated to be 1 in 8 million.

As part of understanding future risk of introduction of *M. bovis*, research is required to understand the true risk posed by imported frozen semen. *M. bovis* DNA was not an uncommon finding in imported semen tested that had been used on the affected enterprise. However, viability was not demonstrated through culture; and effective transmission has yet to be demonstrated.

Overall we may look at the results from the risk pathway analysis as a relative measure of the likelihood of each pathway to lead to an introduction event. However, we can also argue that as only one outbreak has been observed it is impossible to determine which pathway was responsible for this breakdown. It is also possible that some significant event outside of our understanding of risk pathways has occurred and resulted in the outbreak. Thus the possibility of an unforeseen event or series of events e.g. human error should not be ignored.

We should also consider that the characteristics of the affected enterprise are unusual and may have contributed to the epidemiology of the outbreak. Clearly there are some factors that are different from many farms in New Zealand. The enterprise is large scale, on some of the farms utilises robot milking parlours (although not on those farms

determined to be infected), cattle are housed indoors on some farms, and anecdotally the management style adopted in the enterprise is highly entrepreneurial in nature. At face value it is not clear how these differences would necessarily put this enterprise at more risk. An interview with key decision makers for the enterprise found no evidence of an imported conveyer of *M. bovis* that could have explained the introduction of the agent onto the farm enterprise. A further risk factor analysis may be able to provide additional insight.

We also have to consider that the epidemiology of the outbreak on the index farm (within the affected enterprise) was peculiar. The outbreak presented as an unusually high proportion of animals showing clinical signs on the index farm, which might imply a point source epidemic. The classical epidemic from an infectious disease would typically present as a rising increase in the number of cases over time as the number of infected animals and their contacts increased (for instance exponentially or some variant in that level of increase). The presentation on the second 'Infected Property' presented in this classical way as an outbreak of clinical mastitis.

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

Therefore, in summary the pathway analysis undertaken has provided a relative measure of the risks posed from a number of low risk (but non-negligible) pathways. Given that only a single outbreak was identified it is possible that future information may become available which will shed light on the specific pathway that lead to this outbreak occurring.

The work also highlights the value in creating independent multi-expert working groups to tie together different expertise and to combine the multitude of information streams to provide decision-support in complex risk environment.

Based on the above rationale the working group therefore makes the following recommendations:

- Given the knowledge gaps regarding the infection risk from germplasm (i.e. frozen semen and embryos), research funding needs to be allocated to better understand the transmission risk posed by these imported commodities. Thus it is recommended that a research proposal is developed to explore in particular frozen semen as a conveyer of *M. bovis*. This research could include presence and infectivity of *M. bovis* in semen following experimental infection of bulls. The output of this research may be used to inform on the need for and the expected effectiveness of additional risk mitigation steps.
- Locate imported cattle and work up a survey on the status for *M. bovis* in these animals if it is technically feasible (i.e. accounting for things such as availability of an appropriate negative control, duration of *M. bovis* antibodies in relation to import date etc.).

- Continue to suspend live cattle imports until further risk assessment work has been completed.

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Rapid Risk Assessment: *Mycoplasma bovis* in bovine semen

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Rapid Risk Assessment: *Mycoplasma bovis* in bovine semen

September 2017

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Executive summary

This document is a qualitative analysis of the risk posed by *Mycoplasma bovis* (*M. bovis*) in bovine semen.

The methodology for this risk assessment follows the Biosecurity New Zealand *Risk Analysis Procedures- Version 1* (Biosecurity New Zealand 2006). For terrestrial animals these procedures follow the guidelines in the Terrestrial Animal Health Code (hereafter referred to as the *Code*) of the World Organisation of Animal Health (OIE).

The likelihood of *M. bovis* being present in semen is assessed to be low. The likelihood of subsequent exposure and transmission of *M. bovis* to susceptible animals is assessed to be very low but non-negligible. The consequences of entry and establishment of *M. bovis* are assessed to be low.

Mycoplasma bovis is therefore assessed to be a risk in imported bovine semen.

Risk management options have been presented that include the Code's general recommendations for managing artificial insemination centres for general hygiene and for semen collection, processing and storage. As part of the Code's recommendations, the mixture and concentration of bactericidal antibiotics that should be added to the semen is stipulated.

Given the uncertainty associated with the efficacy of standard antibiotic treatments in eliminating *M. bovis* from semen, additional risk management options beyond the international standard are also presented. These options which include testing of semen donors or semen using an MPI approved method for detection of *M .bovis* further reduce the assessed risk associated with *M. bovis* beyond what is achieved by adoption of the international standard. However, the degree to which these measures ameliorate the risk associated with *M. bovis* in semen remains unclear given the uncertainty associated with performance of diagnostic testing.

Introduction

Mycoplasma bovis was identified in a dairy herd in the South Island on the 22nd July 2017. This was the first report of the organism in New Zealand. Following this detection MPI have re-assessed the risk of *M. bovis* associated with the importation of bovine semen and the measures that could be considered to effectively manage this risk.

An import risk analysis was completed in 2009 to assess the risk due to disease-causing organisms associated with the importation of cattle embryos and semen. This risk analysis concluded that the risk estimate for exotic Mollicutes, including *M. bovis*, was non-negligible, and accordingly they were classified as hazards in the commodity. The options presented for the management of risk included:

- Monitor literature to see whether resistance to various antibiotics is reported, and revise the requirements for the antibiotics to be used in semen extender and embryo wash solutions as necessary.
- Culture of germplasm prior to addition of antibiotics. This option would preclude import of product not specifically prepared for New Zealand, i.e. 'on shelf' product.
- Culture of germplasm after addition of antibiotics. This option would be less rigorous than the last but would allow the importation of frozen germplasm that has already been processed and is available "on shelf".

Following a process of internal and external consultation the IHS required:

That the preparation of germplasm be performed in accordance with the recommendations of the OIE Code chapter on collection and processing of bovine semen, and the OIE Code chapter on collection of embryos of livestock, including the use of suitable antibiotics in semen diluents and embryo washing media.

AND

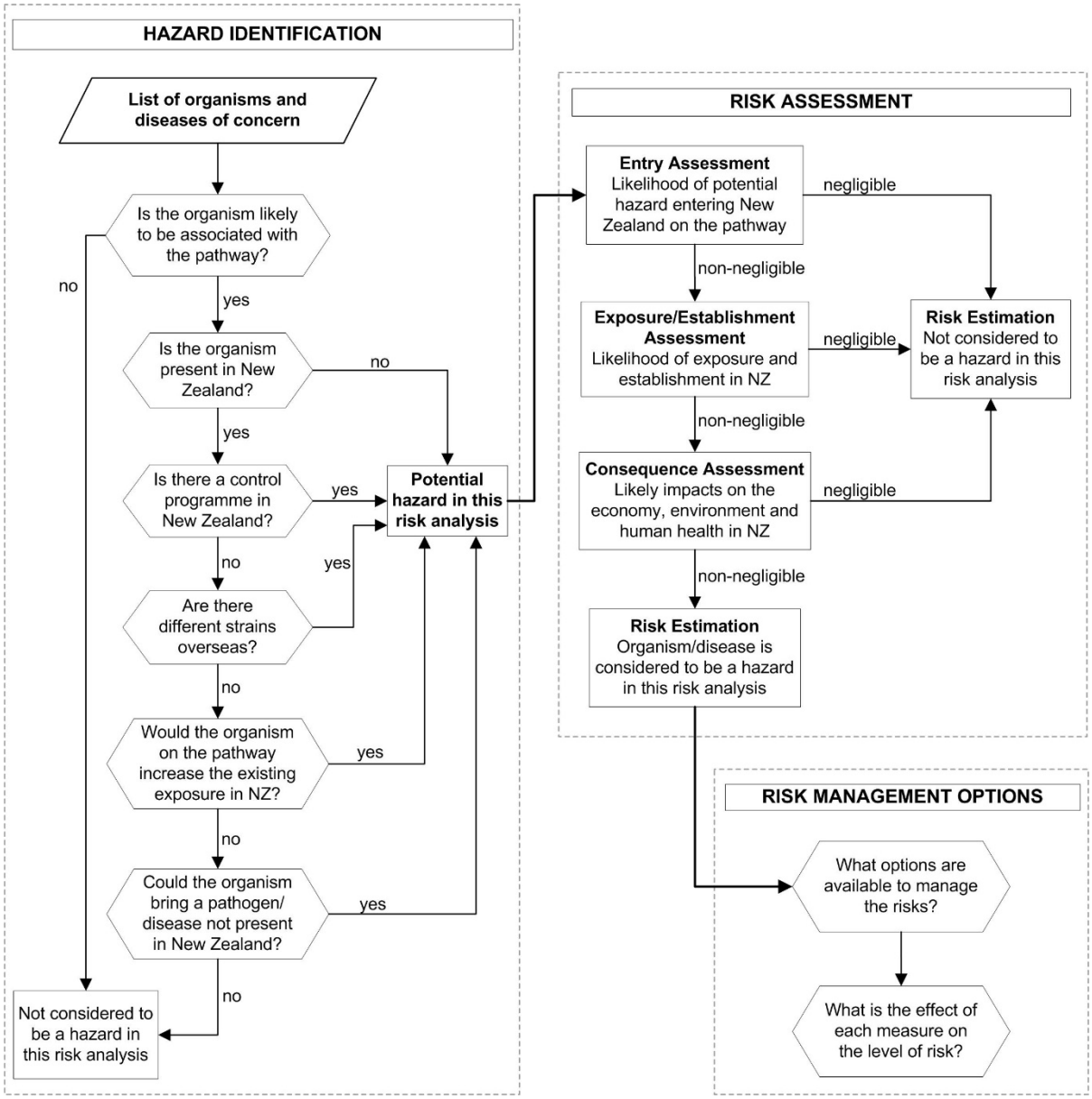
Donors have never recorded a positive test for *M. bovis*.

Scope and commodity definition

This rapid risk assessment qualitatively assesses the risk due to *M. bovis* associated with the importation of bovine semen from approved countries.

Methodology

Figure 1. The risk analysis process.



Mycoplasma bovis

HAZARD IDENTIFICATION

Aetiological agent

Class: Mollicutes Order: *Mycoplasmatales* Family: *Mycoplasmataceae*;
Genus: *Mycoplasma* *Species: Mycoplasma bovis*

OIE list

Mycoplasma bovis (M. bovis) is not an OIE listed disease.

New Zealand status

Up to the 22nd July 2017 *M. bovis* had not previously been detected in New Zealand. However, on this date samples taken from a dairy herd in South Canterbury tested positive for the agent. To date, there are seven farms which have been confirmed positive. Five of these are part of a large dairy farm operation whilst the other two are directly connected by the movement of infected cattle from the infected properties (IPs).

Prior to this detection surveillance for *M. bovis* included passive surveillance and targeted surveillance with surveys performed in 1995 and 2007.

In 1995 a small serological survey was performed using 353 dairy cow serum samples randomly selected from routine submissions to the Central Animal Health Laboratory. Of the 353 samples tested all were negative for antibodies to *M. bovis*. However it was noted at the time that although the sensitivity of the complement fixation test was almost 100% in acute infections, this reduced to 70% and 30% in chronic infections and subclinical cases respectively (Reichel *et. al,* 1999).

In 2007 a random survey of bulk tank milk from dairy herds was performed. A total of 244 bulk milk tank samples were collected and tested by PCR and culture with no detections of *M. bovis*. The study concluded with 99% confidence that *M. bovis* was absent from the national dairy population at a between-herd prevalence of 1.9% (McDonald *et. al,* 2009).

Despite New Zealand's surveillance activities to date it is conceivable that *M. bovis* has been present in New Zealand for a significant period of time but below the detection limit of the Mc Donald *et. al,* study. Both the technical constraints of diagnostic testing and the potential for *M. bovis* to be present at an extremely low prevalence (Nicholas et al 2016) make the demonstration of country freedom particularly challenging.

In addition, an accurate assessment of prevalence should include targeted surveillance of the calf rearing sector given that M. bovis is, in some countries, very much a disease of calves, particularly feedlot, with occasional outbreaks in dairy herds usually from closely sited calves (s 9(2)(a) , personal communication[1]).

1. s 9(2)(a) Consultant, England, email to s 9(2)(a) 13 September 2017.

M. bovis is known to occur worldwide. Prior to the adoption of import health measures in 2006, opportunity for entry of this organism into New Zealand existed via the importation of live cattle.

In view of these live cattle imports, it is conceivable that *M. bovis* was present in New Zealand prior to this current detection (s 9(2)(a) personal communication[1]).

It is biologically possible that *M. bovis* could have been endemic in New Zealand for several years without detection given that delayed infections have previously been observed (s 9(2)(a) personal communication[2]). Furthermore, unless specific mycoplasma identification is carried out or veterinary staff are sufficiently familiar with the clinical and pathological signs of *M. bovis,* then disease can quite easily be mistaken for other bovine respiratory disease particularly with mixed infections (e.g. pasteurella/ mannheimia) (s 9(2)(a) personal communication[1]).

Epidemiology

M. bovis was first isolated in the USA in 1961 and subsequently spread to many countries achieving a worldwide distribution. (Nicholas and Ayling, 2003a). Significant variations in country prevalence of Mycoplasmas are observed globally. Some countries such as Belgium, France, and Greece, have an estimated between herd prevalence of less than 1% to 5.4% (Fox, 2012). In France a study by Arcangioli *et. al*, (2011), designed to estimate a prevalence of *M. bovis* of 2%, with 95% confidence, failed to detect the organism in any of the 345 bulk milk tank samples collected and tested by culture and PCR (Arcangioli *et. al*, 2011).

In contrast to this, surveys performed in Mexico and Iran show significantly higher between herd prevalence estimates of 50-100% (Fox, 2012). Historically high between herd prevalence has been reported in Australia (Ghadersohi *et. al*, 1999). However, it has subsequently become apparent that these earlier reported prevalences were greatly overestimated as a result of the PCR methods used. More recent reports assert that relatively few Australian dairy herds are infected, less than 0.9%, despite the agent being endemic (Morton *et. al*, 2014).

Mycoploasma outbreaks can be highly variable. Sudden mastitis outbreaks associated with high morbidity can be followed by spontaneous elimination. Nicholas *et. al* (2016) noted that the disease is often self-limiting, disappearing within months of outbreaks, sometimes without any intervention.

In New Zealand there have been two reports of explosive outbreaks of mastitis caused by *Mycoplasma alkalescens* in the late 1960s and *Mycoplasma dispar* in the early 1980s respectively (Brookbanks *et. al* 1969; Hodges *et. al* 1983). *M. dispar* has been diagnosed as part of the current outbreak investigation, demonstrating that the agent can be present, presumably at an extremely low level, and not commonly associated with disease.

2. Professor John House BSc BVMS (Hons) PhD, Director Bovine Clinical Services, University of Sydney, Australia, email to J Mounsey 14 September 2017

M. bovis is a recognised cause of respiratory disease, mastitis, arthritis and otitis (Nicholas and Ayling, 2003a). Susceptible animals become infected via inhalation, ingestion or invasion of the teat canal. (Pfutzner and Sache, 1996). Spread of the disease occurs primarily through the movement of infected cattle and the contamination of equipment such as milking machines. A carrier state exists whereby infected animals can continue to shed the organism without clinical signs.

There are limited scientific studies which demonstrate the presence of *M. bovis* in semen or in the male bovine reproductive tract.

In India, Jain et al (2012) collected 22 semen samples from cattle and buffalo. Samples were tested for *M. bovis* using PCR, however the specificity of the PCR test was not reported. Of the 12 semen samples collected from cattle and 10 from buffaloe, *M. bovis* was isolated from 27% and 21% samples respectively.

A study by Khurana and Garg (1996) investigated genital mycoplasmosis in breeding bulls using culture followed by growth inhibition. Mycoplasma species were isolated from 19.7% of 132 preputial samples and 3.9% of 102 frozen semen samples. *M. bovis* was isolated from 1 of the 203 bulls samples, however it is unspecified if this was from a semen or preputial sample.

In a German study Kirchhoff and Binder (1986) collected 182 semen samples and 210 preputial wash samples from normal bulls. *M. bovis* was identified in just one of the preputial samples. The authors also examined two semen samples and one preputial sample from two bulls showing clinical signs of epididymitis, with *M. bovis* isolated from all three samples. *M. bovis* was identified by culture followed by indirect immunofluorescence, however the specificity of the test was not reported.

Trichard and Jacobsz (1985) collected 1005 preputial samples, originating from 5 AI centres and 119 private herds in South Africa and detected *M. bovis* in 6 (0.5%) of 1009 samples. In addition 986 semen samples were collected from 4 AI centres and 112 private herds and *M. bovis* was detected in 5 (0.5%) of 986 samples. In both preputial and semen samples *M. bovigenitalium* occurred most frequently, at 9% and 16% respectively. Samples were subjected to culture followed by direct fluorescent antibody test. The specificity of the test was not reported.

A study by Stripkovits *et. al,* (1983) examined semen samples of 181 bulls originating from four herds for the presence of mycoplasmas and cultured *M. bovis* from 67 of 181 samples. The authors reported a very low level of other mycoplasmas, with only two non- bovis mycoplasmas isolated, indicating that identification to the species level was not accurate. The specificity of the test was not reported.

Langford (1975) cultured semen samples and preputial washes for the presence of *M. bovis* and detected the organism in the semen of four of the 168 bulls sampled and in the preputial washes of four of the 267 bulls sampled. Neither the speciation method used nor the specificity of the test were reported.

Jurmanova & Sterbova (1977) reported the isolation of 56 mycoplasma strains, two of which were identified as *Mycoplasma agalactiae subsp. bovis (M. bovis*). Observed results followed

culture and indirect IF test of 202 semen samples, collected from bulls in regular service for AI in Czecho-Slovakia. The authors observed that mycoplasma positive samples were less motile than those free of the organism.

Several other studies investigating bovine genital mycoplasmosis have evaluated semen and preputial samples for the presence of *M. bovis* and reported no detections of the agent.

In field studies Petit *et. al,* (2008) found that 12.5% of semen samples collected from 273 bulls at five AI centres in Austria had semen contaminated with mycoplasma species, however no *M. bovis* was isolated. Eder-Rohn (1995) detected mycoplasma species in 7.5% of a total of 107 semen samples and reported no isolations of *M. bovis*. Ball et al (1987) examined 332 fresh and 137 processed semen samples and identified mycoplasmas in 23% and 20% of samples respectively, with no detections of *M. bovis*. Garcia *et. al,* (1986) cultured 2950 semen samples from nine Canadian studs, with no detections of *M. bovis.* Fish *et. al,* (1985) showed that 28% of fresh semen samples collected from 45 bulls used for AI had semen contaminated with mycoplasma species, but failed to isolate *M. bovis*. Rae (1982) tested 55 unprocessed semen samples and identified 34 non- bovis mycoplasmas. Erno (1975) reported that 7.8% of semen samples tested were Mycoplasma positive. Of the 158 positive samples 100 were subsequently selected at random for species diagnosis, with 85 identified as *M. bovigenitalium*. No *M. bovis* was detected.

The work of Langford (1975) and Stripkovits *et. al,* (1983) demonstrated the presence of *M. bovis* in semen. Langford (1975) through the detection of *M. bovis* in preputial washes demonstrated how the presence of *M. bovis* in semen is in part due to contamination from the prepuce. However, it remains unclear if *M. bovis* occurs in the ejaculate or if its presence is solely due to contamination.

Fish *et. al,* (1985) investigated the source of mycoplasma species in semen. The semen samples and genital tracts of 45 healthy AI bulls were cultured. The study found that mycoplasma species were most commonly isolated from the prepuce and distal urethra with isolations from testes, epididymides, ampullae, seminal vesicles and proximal urethra occurring infrequently. Furthermore, the study found that in 22 of the 24 semen samples which were positive for mycoplasma species, the same mycoplasma species was subsequently isolated from either the prepuce, the urethral orifice or both of these sites. The authors concluded that the prepuce and the distal urethra are the source of contamination of semen samples with mycoplasma. In the absence of studies which look specifically at *M. bovis* it can only be inferred from the work of Fish *et. al,* (1985) that the male distal reproductive tract is a likely source of contamination of semen with *M. bovis*.

The ability of *M. bovis* to remain viable in semen has been demonstrated experimentally. Hirth *et. al,* (1967) found *M. bovis* remained viable in frozen bull semen for as long as 18 months when added prior to extension and freezing in liquid nitrogen.

Due to specific metabolic and morphological characteristics, mycoplasmas are intrinsically resistant to antimicrobials that interfere with synthesis of folic acid or that act on the cell wall. In addition, mycoplasmas have high mutation rates and can rapidly develop acquired resistance to antimicrobials (Wrathall *et. al,* 2007). Mycoplasmas are generally susceptible to

antibiotics that affect protein (tetracyclines, macrolides, lincosamides, phenicols) or nucleic acid synthesis i.e. fluoroquinolones (Sulyok *et. al*, 2014).

Shin *et. al*, (1988) reported a bactericidal effect of 60-80% for *M. bovis i*n semen using the combination known as GTLS, gentamicin (an aminoglycoside), tylosin (a macrolide), lincomycin (a lincosamide) and spectinomycin (also a lincosamide) at concentrations of 500,100,300 and 600 ug/ml. The authors concluded that although 100% bactericidal effect had not been achieved, the reduction in the number of challenging organisms was significant and that this combination of antibiotics provided effective control of microbial pathogens in semen.

A later study by Visser *et. al*, (1998) also investigated the antibiotic combination of GTLS and its effect on *M. bovis* in frozen bovine semen. It was reported that although GTLS had an obvious bacteriostatic effect no significant bactericidal effect was observed. The authors concluded that this antibiotic combination in semen specimens was not capable of total elimination of the organism in frozen bovine semen.

The OIE code chapter for the collecting and processing of bovine semen continues to recognise the combination of GTLS gentamicin (250 µg), tylosin (50 µg), lincomycin–spectinomycin (150/300 µg) as an antimicrobial combination of acceptable bactericidal activity. However, given the research by Visser, it may be argued, that these antibiotics are at best mycoplasmastatic and at worst largely ineffective for *M. bovis*.

In Europe, several studies investigating *in-vitro* susceptibilities of *M. bovis* have demonstrated increasing resistance to antimicrobials traditionally effective against the organism.

A British study by Ayling *et. al*, (2000) found that oxytetracycline and spectinomycin had a limited effect against the majority of the 62 *M. bovis* field isolates included in the study. Furthermore nearly 20% of the isolates were highly resistant to spectinomycin and tilmicosin was ineffective.

In Hungary, Sulyok *et. al*, (2014) investigated the *in-vitro* antimicrobial susceptibility of *M. bovis* strains collected from nasal swabs and lung tissue. Minimal inhibitory concentrations (MICs) were assessed by broth microdilution. The study demonstrated increasing MICs for tetracyclines and macrolides, indicating increasing resistance to antimicrobials commonly used in the treatment of *M. bovis*. Of significance was the observation that tylosin had a MIC_{90}> 128ug/ml. The OIE recommends the use of tylosin at 50ug/ml as part of the GTLS combination.

Heuvelink et. al, (2016) performed a similar study in the Netherlands, investigating *in- vitro* antimicrobial susceptibility of *M. bovis* isolates originating from lung tissue, mastitic milk and synovial fluid. The highest MIC values were obtained for erythromycin, tilmicosin and tylosin.

All of these studies identified fluoroquinolones as the most efficacious antimicrobial in inhibiting *M. bovis.*

However, increasing resistance to fluoroquinolones as a result of genetic alterations in the form of point mutations within the quinolone resistance-determining regions of *M. bovis* has been described (Lysnyansky & Ayling, 2016).Studies by Mustafa *et. al*, (2013), Lysnyansky

et. al, (2009) and Sato *et. al,* (2013) investigated the susceptibility of *M. bovis* isolates from China, Israel and Japan respectively and demonstrated decreased susceptibility to fluoroquinolones in association with point mutations of the proteins coding for resistance.

It is also of note that the use of Fluoroquinolones, which is considered a critically important antibiotics, to control potential infection is against the WHO/FAO suggestions on good antibiotic stewardship.

Limited research has been completed into the role of infected semen in the transmission of *M. bovis.*

The pathogenicity of *M. bovis* for the bovine reproductive tract has been demonstrated in experimental studies. Hartman *et. al,* (1964) described genital lesions including endometritis, salpingitis and salpingoperitonotis in seven of eight mature virgin heifers following experimental uterine infusion of *M. bovis* (referred to by the author as *Mycoplasma agalactiae* var. *bovis*) whilst Stallheim and Proctor (1976) reported placentitis, fetal deaths and abortions following intrauterine inoculation.

Hirth *et. al,* (1966) investigated the potential of infected frozen semen as an agent of transmission. Twelve heifers were inseminated with frozen semen, to which *M. bovis* had been added. Although an antibody response was demonstrated in some heifers it is difficult to interpret its significance given that the author notes that results were inconsistent and false positives were a problem. Cervico-vaginal mucus samples were collected throughout the study with results showing that of the 12 heifers inseminated with semen containing *M. bovis* 12, 6 and 1 heifer(s) were culturally positive at week 8, 20 and 32 respectively. Four of the 12 heifers inseminated with *M. bovis* delivered live calves which were clinically normal and *M. bovis* was not isolated from the calf or the dam at parturition. Eight heifers were necropsied, with varying degrees of chronic suppurative salpingitis, chronic endometritis and ovarian adhesions observed in four and no significant changes observed in the remaining four.

These experimental studies demonstrate the pathogenicity of *M. bovis* for the female reproductive tract. In addition, Hirth *et. al,* (1966) demonstrated that heifers exposed to *M. bovis* in semen may act as a source of the bacteria by shedding the organism in cervico-vaginal mucus for extended periods. The viability of this potential route of transmission to other susceptible animal through direct contact has not been investigated.

There are no field studies to demonstrate that naturally occurring *M. bovis* in semen can transmit disease to susceptible heifers or cows. Furthermore, there are no evidence based reports in the literature where infected semen is definitively demonstrated to be the route by which incursions have occurred.

It has been speculated that semen may have been responsible for the introduction of *M. bovis* into the UK (Wrathall et al 2007) and into Finland (Neilsen, 2016). However these claims remains unsubstantiated and are further diminished when one considers the significant number of live cattle imports into these countries from infected regions, a well -recognised and proven mode of introduction.

Additionally, unpublished data from Finland has reported the suspected introduction of *M. bovis* infection into a closed dairy herd via AI (s 9(2)(a), personal communication[3]). The case report details the occurrence, in 2015, of *M. bovis* mastitis following insemination of cows with *M. bovis* contaminated semen. Although this report presents an apparent case for a

venereal route there is insufficient evidence to prove cause and effect. Given that *M. bovis* has been endemic in Finland for a number of years, first detection reported in 2012, other routes of introduction to this herd are plausible.

Hazard identification conclusion

Mycoplasma bovis can be present in the semen of bulls .The organism has been described as a cause of respiratory disease, mastitis, arthritis and otitis

It is concluded that *M. bovis* is considered a potential hazard in the commodity.

RISK ASSESSMENT

Entry assessment

Of the 13 studies identified for this review which evaluated whether semen could be infected with *M. bovis*, 4 identified *M. bovis* in semen from normal bulls and 1 identified the agent in the semen of bulls with epididymitis. In most cases the proportion of positive samples was very low, less than 2.5%. In the two studies (Jain *et al* 2012; Stripkovits *et al* 1983) which reported a high prevalence of *M. bovis* in semen, 36% and 37% respectively, it is likely that the reported prevalence was inaccurate (s 9(2)(a), personal communication[4]). Jain et al (2012) used a PCR which had no data on specificity or sensitivity whilst Stripkovits et al (1983) reported a very low level of other mycoplasmas, indicating that identification to the species level was not accurate.

These same field studies have also shown that semen from donors bulls can be contaminated with *M. bovis* in the absence of clinical signs. Once present in semen *M. bovis* can survive for prolonged periods and is not eliminated by processing or freezing (Hirth 1967). It has been demonstrated that the antibiotics commonly used in semen extenders may not be completely effective against *M. bovis* in semen in all cases.

The isolation of *M. bovis* in semen has been demonstrated infrequently. However, once present in semen *M. bovis* can withstand processing, freezing and certain antibiotic treatments. Accordingly, the likelihood of entry is assessed to be low but non-negligible.

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Exposure assessment

The likelihood of exposure is certain since imported semen is inseminated into susceptible females. However, significant uncertainty relates to the likelihood of transmission should *M. bovis* be present in semen.

There are no reports in the published literature to demonstrate that naturally occurring *M. bovis* in semen can transmit disease to susceptible heifers or cows. Furthermore, there are no published accounts where infected semen has been proven to be the route by which introduction of the agent has occurred.

However, it is unknown whether semen would be accurately identified as the source of an outbreak should it occur. Both the endemic nature of *M. bovis* in all cattle-rearing countries (s 9(2)(a) , personal communication[1]) and the potential lag between the use of the semen and clinical diagnosis could potentially pose difficulties in proving semen as the source of infection. Thus limited conclusions can be drawn from the lack of published evidence demonstrating *M. bovis* contaminated semen as a route of transmission s 9(2)(a) , personal communication[2]).

The pathogenicity of *M. bovis* for the reproductive tract of the cow has been demonstrated in experimental studies (Hartmann *et. al,* 1964; Hirth *et. al,* 1966). There are no published reports demonstrating systemic infections such as mastitis, pneumonia or arthritis resulting from experimental infections with *M. bovis* contaminated semen. The correlation between the artificial dose of *M. bovis* used in these studies and the level of *M. bovis* in naturally infected semen in unknown and as such the experimental studies provide only very limited support for the likelihood of transmission of *M. bovis* by semen.

Nevertheless, it may be hypothesised that once *M. bovis* is in the blood stream at the required infectious dose there is no practical obstacle to haematogenous spread and subsequent infection of the udder, or to a lesser degree given the higher infectious dose required, the lungs s 9(2)(a) , personal communication[1]).

Experimental studies have demonstrated the ability of *M. bovis* to reproduce naturally within the female reproductive tract and to be present in cervico-vaginal mucus. Notably, this ability of *M. bovis* to colonise the female reproductive tract following insemination with *M. bovis* infected semen has only been demonstrated experimentally (Hirth 1966).

Despite this, it may be hypothesised that infection via contaminated semen could result in multiplication of the organism within the female reproductive tract followed by spread from the initially infected cow to other animals.

In summary, there is limited experimental evidence demonstrating the pathogenicity of *M. bovis* for the reproductive tract. There is no evidence to demonstrate that transmission of *M. bovis* via semen can occur naturally. Internationally traded semen exposed to recipient animals is not a recognised pathway for disease transmission and has never been demonstrated. However, given the challenges associated with demonstrating contaminated semen as a source of natural infection, limited conclusions can be drawn from this absence of evidence.

The likelihood of *M. bovis* transmitting to an exposed recipient is highly uncertain. There has been inadequate study of this particular pathway and consequently a paucity of scientific data.

On the basis of currently available scientific evidence the likelihood of transmission is assessed to be very low but non-negligible.

Consequence assessment

Although it is generally thought that *M. bovis* is very host specific to cattle, there are infrequent rare reports of *M. bovis* in hosts such as sheep, goats and deer (Kumar et. al, 2012; Ayling *et. al,* 2004; Egwu *et. al,* 2001; Dyer *et. al,* 2004).

Therefore, the consequences of *M. bovis* are limited to the dairy and beef industries only. *M. bovis* impacts the health and production of cattle herds, thereby causing economic losses. Production losses including reduced milk production and increased culling, as a result of therapy resistant mastitis, and reduced daily weight gain due to calf pneumonias and arthritis are observed in affected herds.

Since New Zealand cattle are not housed indoors over winter it is more likely that consequences would be similar to Australia's paradigm, rather than that of the US or Canada's for instance. It is therefore probable that a very low level of prevalence with little impact to the industry as a whole could be expected.

Indeed, *M. bovis* is not recognised by the OIE as a significant disease of concern to trade. Thus the market eligibility for bovine products and the export of live cattle and bovine germplasm is currently assumed to not be affected by the identification of *M. bovis* in New Zealand. Therefore, there are limited consequences to the trade of live animals or animal products.

s 6(a), s 9(2)(d) [redacted]

In conclusion, there are likely to be very limited consequences for trade. There is a very rare possibility for s 6(a), s 9(2)(d) [redacted] the health of sheep, goats and deer. It is only for the cattle industries that consequences in regards animal health and production losses could occur. These are likely to be similar to that experienced in Australia. Accordingly, the consequences are assessed to be low.

Risk estimation

Since the entry, exposure and consequence assessments are non-negligible, the risk is estimated to be non-negligible and *M. bovis* is assessed to be a risk in imported semen. Consequently, risk management measures can be scientifically justified.

RISK MANAGEMENT

The following points were taken into account when describing options for effectively managing the risks:

- *M. bovis* has been isolated in semen
- *M. bovis* in semen most likely occurs as a result of contamination from the distal urethra and prepuce.
- Antibiotics alone are unlikely to be effective in eliminating *M. bovis* from semen.
- Experimentally *M. bovis* has been demonstrated to be pathogenic for the bovine female reproductive tract
- Experimentally *M. bovis* has been shown to colonise the bovine female reproductive tract and can be isolated in cervical mucus for up to 8 months post exposure
- Field studies have not demonstrated transmission of *M. bovis* following AI with infected semen
- Internationally traded semen has not been demonstrated as a transmission pathway for *M. bovis (*despite a long standing global trade of several hundred thousand straws annually in New Zealand).
- If semen transmission was a frequent international event it is assumed that more infections with a diversity of strains would be seen in different countries s 9(2)(a) personal communication[2])
- *M. bovis* is now confirmed in New Zealand following a clinical outbreak etc. in the Canterbury region but the geographical extent to which infection is currently present in New Zealand is unknown.

Options

Option 1

Semen from donor bulls must be collected, handled, prepared, processed and stored in accordance with chapters 4.5 and 4.6 of the OIE Code

This option would likely significantly reduce but not eliminate what is judged to be a low probability of *M. bovis* being present in semen, and consequential transmission.

Option 2

Semen from donor bulls must be collected, handled, prepared, processed and stored in accordance with chapters 4.5 and 4.6 of the OIE Code

Donors have never recorded a positive test for *M. bovis*.

This is the current measure in place in New Zealand

This option would likely further reduce the probability of infected semen over and above that achieved by the OIE Code provisions alone. The extent of this on further reducing the risk of transmission is unknown.

Option 3

Semen from donor bulls must be collected, handled, prepared, processed and stored in accordance with chapters 4.5 and 4.6 of the OIE Code

Testing of semen donors using an MPI approved test.

This option would likely further reduce the probability of infected semen over and above that achieved by the OIE Code provisions alone. The extent in further reducing the risk of transmission is unknown, however it would be expected that a validated and approved test would be an enhancement over a non-specified test as above.

However due to the constraints of diagnostic testing of live animals this measure would not entirely eliminate the low probability of *M. bovis* in semen, and subsequent risk of transmission. The ELISA test is validated as a herd detection assay with an estimated sensitivity of approximately 75%. Testing of individual animals rather than the herd is problematic in that individual animal titres are poorly correlated with infection or disease i.e. not all infected animals will develop high antibody titres.

Option 4

Semen from donor bulls must be collected, handled, prepared, processed and stored in accordance with chapters 4.5 and 4.6 of the OIE Code

Testing of semen using an MPI approved method of detection for *M. bovis.*

This option also would likely further reduce the probability of infected semen over and above that achieved by the OIE Code provisions alone. The extent in further reducing the risk of transmission is unknown, however it would be expected that a validated and approved test would be a risk-reduction enhancement.

If taken as an option, validation of testing methods would be required. At present, information relating to the analytical and diagnostic performance of such tests is incomplete. Furthermore it has been suggested that variability between batches of semen from the same animal, and between straws of semen from the same batch may exist.

With regard to options 2, 3 and 4:

i) In order to meet the requirements of The Agreement on the Application of Sanitary and Phytosanitary Measures, hereafter referred to as the SPS agreement, the adoption of a higher level of protection than what is currently afforded by the OIE Code would have to be supported by robust epidemiological evidence demonstrating zone or country freedom from *M. bovis*.

ii) The adoption of a higher level of protection than what is afforded by the OIE Code could restrict the use of imported semen. If this was considered as a risk management option, an assessment of the impact of any potential loss of genetic gain is recommended.

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