Biosecurity New Zealand
Tiakitanga Pūtaiao Aotearoa

Rapid Risk Assessment

Mycoplasma bovis in bovine semen

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New Zealand Government

Ministry for Primary Industries
Manatū Ahu Matua

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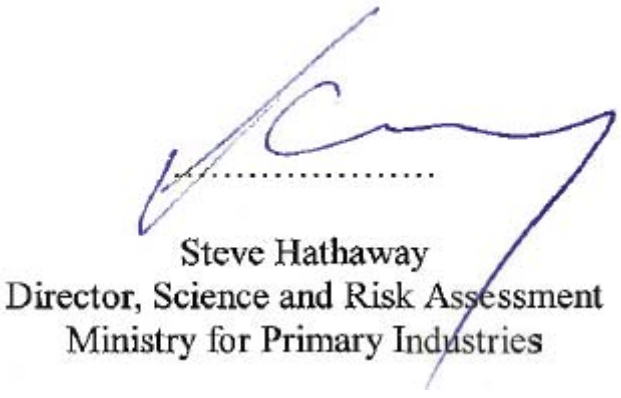
Rapid Risk Assessment: *Mycoplasma bovis* in bovine semen

Version 2.0

February 2019

Approved for IHS development



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Version information

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1.0	First peer-reviewed version	September 2017
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New Zealand is a member of the World Trade Organisation and a signatory to the Agreement on the Application of Sanitary and Phytosanitary Measures ("The Agreement"). Under the Agreement, countries must base their measures on an International Standard or an assessment of the biological risks to plant, animal or human health.

This document provides a scientific analysis of the risks of *Mycoplasma bovis* in bovine semen. It assesses the likelihood of entry, exposure, establishment and spread of this agent in relation to imported semen and assesses the potential impacts of this organism should it enter and establish in New Zealand. The document has been internally and externally peer reviewed.

Contents

		Page
1.	Executive summary	2
2.	Introduction	3
3.	Scope and commodity definition	3
4.	*Mycoplasma bovis*	7

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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. The direct consequences of the entry and establishment of *M. bovis* for the beef and dairy cattle industries are assessed to be high, both in terms of production losses and resultant economic losses. The indirect consequences of the entry and establishment of *M. bovis* for the economy (trade and market access) are assessed to be low, and for society as a result of control and eradication activities, are assessed to be moderate.

The direct consequences of the entry and establishment of *M. bovis* to the health of humans is assessed to be extremely low. Direct and indirect consequences of the entry and establishment of *M. bovis* in non-bovine species, is assessed to be very low.

The overall consequence assessment has been assessed as moderate.

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 that the efficacy of standard antibiotic treatments in eliminating *M. bovis* from semen is not well established, 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* are likely to further reduce the assessed risk associated with *M. bovis* beyond what is achieved by adoption of the international standard but the level of any such reduction is unknown.

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*.

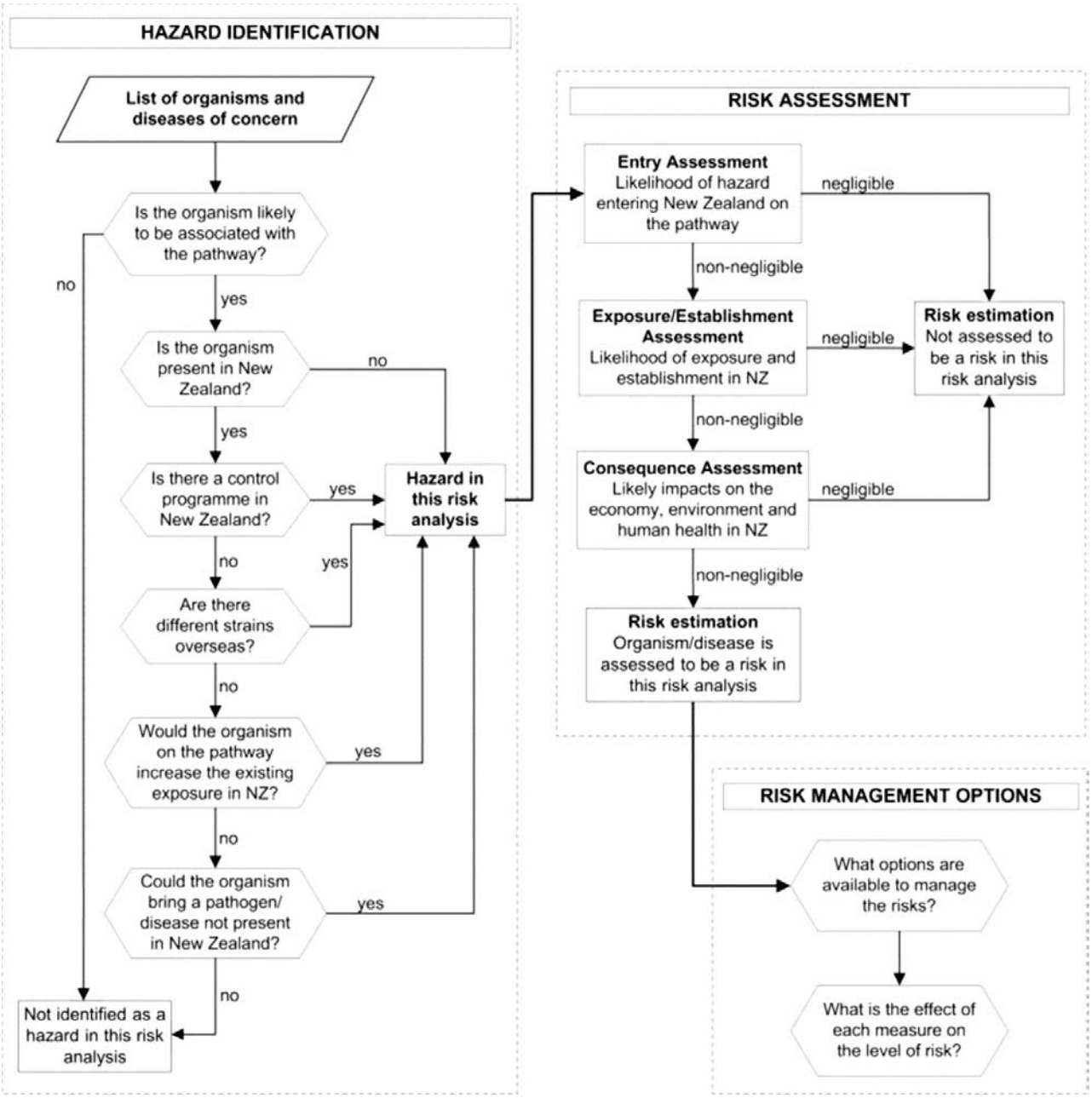
Scope and commodity definition

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

Risk analysis methodology

The methodology used in this risk analysis follows the guidelines as described in *Biosecurity New Zealand Risk Analysis Procedures – Version 1* and in Chapter 2.1 of the *OIE Code* (2018). The process followed is shown in Figure 1.

Figure 1. The risk analysis process



Hazard Identification

Hazard identification includes formal identification of the organism (potential hazard associated with the commodity), whether it is the cause of an OIE listed disease, its New Zealand status, and a discussion on the epidemiology and characteristics of the organism and the disease. The hazard identification section is concluded by a determination of whether the organism is identified as a hazard or not. If the organism is identified as a hazard, it is subjected to risk assessment.

Risk Assessment

Risk assessment consists of:

a) *Entry assessment*: The likelihood of a hazard (pathogenic organism) being imported with the commodity.

b) *Exposure assessment*: Describes the biological pathway(s) necessary for exposure of susceptible animals or humans in New Zealand to the hazard and the ability for the organism/disease to establish and spread in the country.

c) *Consequence assessment*: Describes the likely potential consequences of entry, exposure and establishment or spread of an imported hazard.

d) *Risk estimation*: An estimation of the risk posed by the hazard associated with importing products. This is based on the entry, exposure and consequence assessments. If the risk estimate is assessed to be higher than negligible (i.e. High, Moderate, or Low) then the hazard is assessed to be a risk and risk management measures may be justified to reduce the level of risk to an acceptable level.

Not all of the above steps may be necessary in all risk assessments. The OIE methodology makes it clear that if the likelihood of entry is negligible for a certain hazard, then the risk estimate is automatically negligible and the remaining steps of the risk assessment need not be carried out. The same situation arises when the likelihood of entry is non-negligible but the exposure assessment concludes that the likelihood of susceptible species being exposed is negligible, or when both entry and exposure are non-negligible but the consequences of introduction are assessed to be negligible.

Risk Management

For each organism assessed to be a risk, options are identified for managing that risk. Recommendations for the appropriate sanitary measures to achieve the effective management of risks are not made in this document. These will be determined when the IHS and risk management proposal documents are drafted.

As obliged under Article 3.1 of the World Trade Organization's Agreement on the application of Sanitary and Phytosanitary measures (the SPS agreement) the measures adopted in IHSs will be based on international standards, guidelines and recommendations where they exist except as otherwise provided for under Article 3.3. That is, measures providing a higher level of protection than international standards can be applied if there is scientific justification, or if there is a level of protection that the member country considers is more appropriate following a risk assessment.

Risk Communication

After a draft import risk analysis has been written, MPI analyses the options available and proposes draft measures for the effective management of the identified risks. These are then presented in a draft Import Health Standard (IHS) that is released for public comment, and provides a link to the draft risk analysis.

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.

M. bovis in cattle

Mycoplasmas are reported to cause chronic disease with a high morbidity and low mortality. They also have a long incubation period with subclinical carriers and are difficult to detect via current testing methods. Mycoplasmas persist in the face of microbial therapy and the absence of effective vaccines cause significant problems in diagnosis and control (Wawegama & Browning, 2017). Although large numbers of *M. bovis* can be isolated from clinical cases, low levels or none are found in carriers and chronically infected cattle (Jasper 1981). Negative results are likely in such cases, as well as in cultures of bulk tank-milk samples because of the intermittent shedding of *M. bovis* by infected cattle and the effects of dilution when only small numbers of animals in a milking herd are shedding (Jasper *et. al*, 1979; González *et. al*, 1986).

New Zealand status

Up to the 22 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.

At the time of writing this report evidence of infection had been found in both the North and South Islands (dairy and beef cattle). The current disease management strategy is to attempt to eradicate *Mycoplasma bovis* (MPI 2018).

Prior to the detection, *M. bovis* was included in passive surveillance programs, however, routine exotic disease investigations carried out continuously as part of New Zealand's passive surveillance system had not detected the organism.

New Zealand conducted two targeted surveillance programs in 1995 and 2007 in the dairy sector. No testing was conducted in the beef sector at that stage.

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* using the complement fixation test. 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 national dairy herds was performed. A total of 244 bulk tank milk samples were collected and tested using a nested *M. bovis* PCR, and

bacteriological culture employing enrichment in mycoplasma broth and direct plating onto mycoplasma agar 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 up to July 2017, it is conceivable that *M. bovis* had been present in New Zealand for a significant period of time but below the detection limit of the Mc Donald *et. al,* (2009) 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 feedlots, with occasional outbreaks in dairy herds usually acquired from closely sited calves (Nicholas, personal communication[1]). MPI has included sampling and testing of calf rearing properties as part of the phased eradication of *M. bovis* (BNZ 2018).

Prior to the adoption of import health measures in 2011, opportunity for entry of this organism into New Zealand existed via the importation of live cattle (Nicholas, personal communication[1]). However, in relation to the source of the current outbreak, the absence of recent cattle imports along with the genetic analysis suggests that this pathway is less plausible than others (McFadden *et. al*, 2017).

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 (House, 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*, the disease can quite easily be mistaken for other bovine pneumonia, mastitis and or arthritis, particularly with mixed infections (Nicholas, personal communication[1], Pfutzner & Sachse, 1996).

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 prevalence of mycoplasma mastitis are observed globally. Some countries such as Belgium, France, and Greece, have an estimated between herd prevalence of less than 1% to 5.4% for *M. bovis*, based on bulk tank surveys (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 investigating mycoplasma mastitis indicate between herd prevalence estimates as high as 55-100% (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

[1] Dr R A J Nicholas MSc, PhD, FRCPath, Consultant, England, email to J Mounsey 13 September 2017.

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

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.

Mycoplasma 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 buffalo, *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 preputial samples, originating from 5 artificial insemination (AI) centres and 119 private herds in South Africa and detected *M. bovis* in 6 (0.5%) of the 1099 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 the 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.

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 immunofluorescent 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.

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.

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.

As previously illustrated, studies have demonstrated the presence of *M. bovis* in semen. Additionally, through the detection of *M. bovis* in preputial washes studies have also 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.

M. bovis has also been isolated in commercial frozen semen at the Israeli Company for Artificial Insemination and Breeding (Amram *et. al,* 2013).

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* in 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 µg/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 50 µg/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 antibiotic, 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 infectivity 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 salpingoperitonitis 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 infectivity 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 animals through direct contact has not been investigated.

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).

M. bovis was detected in Finland for the first time in 2012. In 2015 mastitis outbreaks were reported in two closed and adequately biosecure herds. Haapala *et. al,* (2018) describes how AI with *M. bovis* contaminated semen was the source of the outbreaks in both herds. Semen lots from the donor bull were confirmed positive for *M. bovis* following PCR analysis and

culture. This is the first study to demonstrate processed semen used in AI as a source of *M. bovis* infection on a farm.

Presently, there are no prescribed tests for *M. bovis* for international trade. Current detection methods include culture, molecular and serological detection (Wawegama & Browning, 2017). Milk, joint fluid, bronchiolar lavages, swabs (from different anatomical sites), serum samples (Calcutt *et. al,* 2018), semen or embryos (Bielanski *et. al,* 2000) may be tested. However, information on the performance characteristics of such tests is lacking.

Hazard identification conclusion

Mycoplasma bovis is identified as a potential hazard in the semen of bulls.

RISK ASSESSMENT

Entry assessment

Of the 13 studies identified for this review, four identified *M. bovis* in semen from normal bulls and one 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 (Laven, personal communication[3]). 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 donor 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 (Haapala *et. al,* 2018) 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.

[3] Professor Richard Laven BVetMed PhD MRCVS, Associate Professor (Production Animal Health) Massey University, New Zealand, email to J Mounsey 18th September 2017

Exposure assessment

The likelihood of exposure is high since the semen from one infected bull can result in the production of numerous semen straws that may be inseminated into many susceptible heifers or cows, making exposure higher in comparison to embryos. However, significant uncertainty relates to the likelihood of transmission should *M. bovis* be present in semen.

The study by Haapala *et. al,* (2018) describes how AI with *M. bovis* contaminated semen was the source of mastitis outbreaks in two herds in Finland.

Accurately identifying semen as a source of *M. bovis* outbreaks has been considered a difficult task. Both the endemic nature of *M. bovis* in all cattle-rearing countries (Nicholas, 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. However, in the Finnish study, it was possible to identify transmission in a country where both *M. bovis* prevalence and cattle density are low.

The infectivity of *M. bovis* for the reproductive tract of the cow has been demonstrated in experimental studies (Hartmann *et. al,* 1964; Hirth *et. al,* 1966). Evidence of mastitis outbreaks in two herds as a result of AI with *M. bovis* contaminated semen has been published (Haapala *et. al,* 2018). The correlation between the artificial dose of *M. bovis* used in these studies and the level of *M. bovis* in naturally infected semen is 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 (Nicholas, 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 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 some experimental evidence demonstrating the infectivity of *M. bovis* for the reproductive tract. Internationally traded semen exposed to recipient animals is not a recognised pathway for disease transmission and has never been demonstrated.
The likelihood of *M. bovis* transmitting to an exposed recipient has been proven in the Haapala *et, al*. (2018) study. The semen from one infected bull could result in the production of numerous semen straws and can be inseminated into many susceptible heifers or cows. On

the basis of currently available scientific evidence the likelihood of transmission is assessed to be very low.

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). However, the consequences of *M. bovis* are limited to the dairy and beef industries. *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, reduced daily weight gain due to calf pneumonias and arthritis are observed in affected herds.

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 detection of *M. bovis* in New Zealand.

M. bovis is not a recognised pathogen of humans and it is not known to be a food safety risk (MOH, 2017). There are just two reported cases in the literature of *M. bovis* isolation in humans who were immunocompromised (Madoff *et. al,* 1979; Pitcher and Nicholas, 2005).

The consequences for trade following the entry and establishment of *M. bovis* are likely to be very limited; there is a very rare likelihood of potential consequences for human health and the health of sheep, goats and deer.

When considering the impact to the cattle industries it was acknowledged that *M. bovis* impacts the health and production of cattle herds, thereby causing economic losses. In the early stages of *M. bovis* detection, it was expected pastoral-based farming systems adopted by New Zealand would to some degree limit the impact of the disease and that the consequences in terms of animal health and production losses would be similar to Australia's situation, rather than that of the US or Canada's for instance.

However, this assumption is challenged by the epidemiology of the disease observed on 1 of the 2 initially infected properties. A rapid spread of disease was observed on this premises despite it being a farm which utilises traditional pasture feed systems over the winter and while the herd was dry. Currently MPI are conducting an impact study on the effects of *M. bovis* on infected farms. This should provide a clearer understanding of the disease within the New Zealand scenario.

A report produced by Dairy NZ, with the support of Fonterra and DCANZ (Dairy Sector Economic Impact Analysis for *M. bovis*, completed 27th September 2017) highlights fundamental differences between the New Zealand and Australian farming systems, which challenge any assumption that the consequences in terms of animal health and production losses likely to occur in New Zealand would be similar to that observed in Australia. A summary of these differences include:

- Share milking arrangements are not used in Australia in comparison to New Zealand's regular movement of herds during share milking.
- The closer proximity of dairy farms to each other in New Zealand is far greater i.e. in Australia land use is more diverse.

- In the New Zealand system off-farm grazing is used where most young stock less than 2 years of age, and many adult cattle during the non-lactating (dry) period are grazed off the dairy platform.

Since the first detection in the South island, surveillance has shown that the agent has spread across both North and South islands. The first two infected properties (IPs) in South Island were significantly impacted with clinical disease consistent with *M. bovis*. Clinical signs included dry and lactating cow mastitis, arthritic lesions and non-responsive mastitis (Hay 2017). A third IP showing clinical signs was identified in the South Island. Clinical signs included non-responsive mastitis (Barclay, personal communication[4]). As New Zealand's current disease management strategy is to attempt to eradicate *M. bovis* (MPI 2018) in the cattle (beef and dairy) population, it is expected that there will be impacts associated with specific disease control activities related to eradication such as movement restrictions, and culling of infected animals leading to economic losses and restricted farming. Losses of animals (and associated genetics) and losses of livelihood will also result in significant emotional and financial stress to farmers and their families.

The direct consequences of the entry and establishment of *M. bovis* for the beef and dairy cattle industries are assessed to be high, both in terms of production losses and resultant economic losses. The indirect consequences of the entry and establishment of *M. bovis* for the economy (trade and market access) are assessed to be low, and for society as a result of control and eradication activities, are assessed to be moderate.

The direct consequences of the entry and establishment of *M. bovis* in the health of humans is assessed to be extremely low. Direct and indirect consequences of the entry and establishment of *M. bovis* in non-bovine species, are assessed to be very low.

The overall consequence assessment is moderate.

Risk estimation

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

RISK MANAGEMENT

The following information was taken into account when describing options for 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 infective for the bovine female reproductive tract

[4] Dr. Alix Barclay, Mycoplasma bovis 2017 Intelligence Group Manager, Biosecurity New Zealand, MPI, skype business call to K. Govender on 27 November 2018

- 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
- Research studies have not demonstrated transmission of *M. bovis* following AI with infected semen
- Evidence of mastitis outbreaks in two Finnish herds as a result of AI with *M. bovis* contaminated semen has been published (Haapala *et. al,* 2018)
- Internationally traded semen has not been identified 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 (House, personal communication[2])
  M. bovis has been confirmed in New Zealand following a clinical outbreak in the Canterbury region. At the time of writing this report, evidence of infection had been found in both the North and South Islands (dairy and beef cattle).

Options

Option 1

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

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

Option 2

Semen from donor bulls is 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 may further reduce the probability of infected semen over and above that achieved by the OIE Code provisions alone.

Option 3

Semen from donor bulls is 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 may further reduce the probability of infected semen over and above that achieved by the OIE Code provisions alone. It would be expected that application of an approved test would be an enhancement over a non-approved test if that was the test chosen in Option 2 but this would still not eliminate the chance of transmission e.g. the ELISA test is validated as a herd detection assay with an estimated sensitivity of approximately 75% (AHL, personal communication[5]), and testing of individual animals rather than the herd is problematic since not all infected animals will develop detectable antibody titres.

Option 4

Semen from donor bulls is 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 may further reduce the probability of infected semen over and above that achieved by the OIE Code provisions alone.

Comment

There is very little information available on the quantitative diagnostic performance of the tests in Options 2, 3 and 4 as described above. Until such information is available, their relative performance cannot be compared.

There is likely to be some further reduction in the likelihood of entry when any of these tests are applied over and above the Code provisions but this further reduction cannot be quantified.

Validation and MPI approval of a test would establish likely performance characteristics that would assist in assessing the level of risk reduction achieved and would also provide for consistent and repeatable outcomes from routine application.

[5] National Animal Health Laboratory (AHL), MPI, Wallaceville, Upper Hutt, Wellington, email to K. Govender 14 August 2018

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