Biosecurity New Zealand
Tiakitanga Pūtaiao Aotearoa

Rapid Risk Assessment

Mycoplasma bovis in bovine *in-vivo* derived and *in-vitro* produced embryos

Prepared for Ministry for Primary Industries by
Animals and Aquatics,
Science and Risk Assessment,
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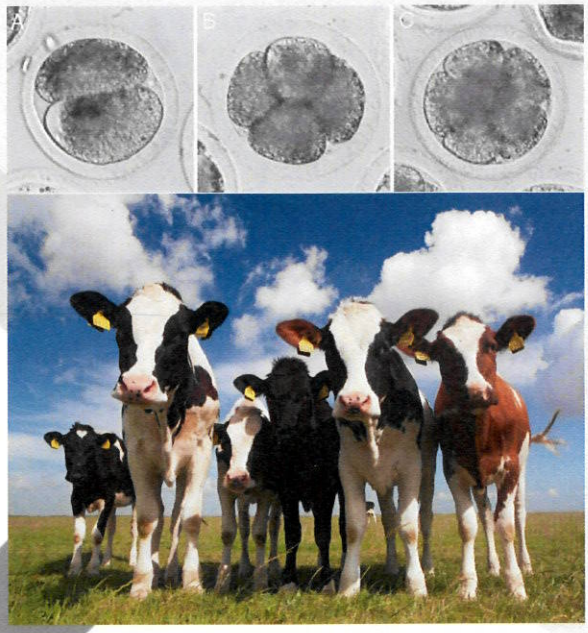
Ministry for Primary Industries



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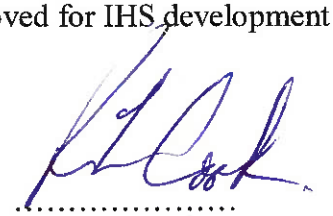
Rapid Risk Assessment: *Mycoplasma bovis* in bovine *in-vivo* derived and *in-vitro* produced embryos

Version 1.0

December 2018

Approved for IHS development



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Version information

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1.0	First peer-reviewed version	December 2018

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 *in-vivo* derived and *in-vitro* produced embryos. It assesses the likelihood of entry, exposure, establishment and spread of this agent in relation to imported *in-vivo* derived and *in-vitro* produced embryos 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.

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Executive summary

This document is a qualitative analysis of the risk posed by *Mycoplasma bovis* (*M. bovis*) in bovine *in-vivo* derived and *in-vitro* produced embryos.

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 for Animal Health (OIE).

The likelihood of *M. bovis* being present in *in-vivo* derived or *in-vitro* produced embryos is assessed to be very low. The likelihood of subsequent exposure and transmission of *M. bovis* to susceptible animals is assessed to be very low but non-negligible. The overall consequence of entry and establishment of *M. bovis* is assessed to be moderate based on impacts to the dairy and beef industry, the current control and eradication costs for this disease and indirect effects on communities. *M. bovis* is therefore qualitatively assessed to be a risk in imported bovine embryos.

Risk management options have been presented that include the *Code*'s general recommendations for embryo collection, processing and storage.

Given that *in-vivo* derived and *in-vitro* produced embryos can retain viable *M. bovis* despite standard processing procedures including washing of embryos in accordance with International Embryo Technology Society (IETS) protocols, trypsin treatment and exposure to antibiotic combinations, additional risk management options beyond the international standard are also presented. These options include testing of embryo donors or testing of embryos (or appropriate samples such as oocytes, co-culture cells) using a Ministry for Primary Industries (MPI) approved method for detection of *M. bovis* and are likely to further reduce the risk associated with *M. bovis* beyond what is achieved by adoption of the international standard. However, the degree to which these measures further reduce the risk associated with *M. bovis* in embryos remains unclear given the uncertainty associated with performance of diagnostic testing.

Introduction

An import risk analysis was completed in 2009 to assess the risk due to disease-causing organisms associated with the importation of bovine *in-vivo* derived 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 risks 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 Import Health Standard required:

That the preparation of germplasm be performed in accordance with the recommendations of the OIE Code chapter 4.6 on collection and processing of bovine, small ruminant and porcine semen, and the OIE Code chapter 4.7 on collection and processing of embryos of livestock and equids, including the use of suitable antibiotics in semen diluents and embryo washing media.

AND

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

M. 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. This Rapid Risk Assessment [1](RRA) was published in September 2017.

The risk management options presented in the 2017 RRA for bovine semen are as follows:

- Semen from donor bulls is collected according to OIE Code recommendations
- Donors have never recorded a positive test for *M. bovis*

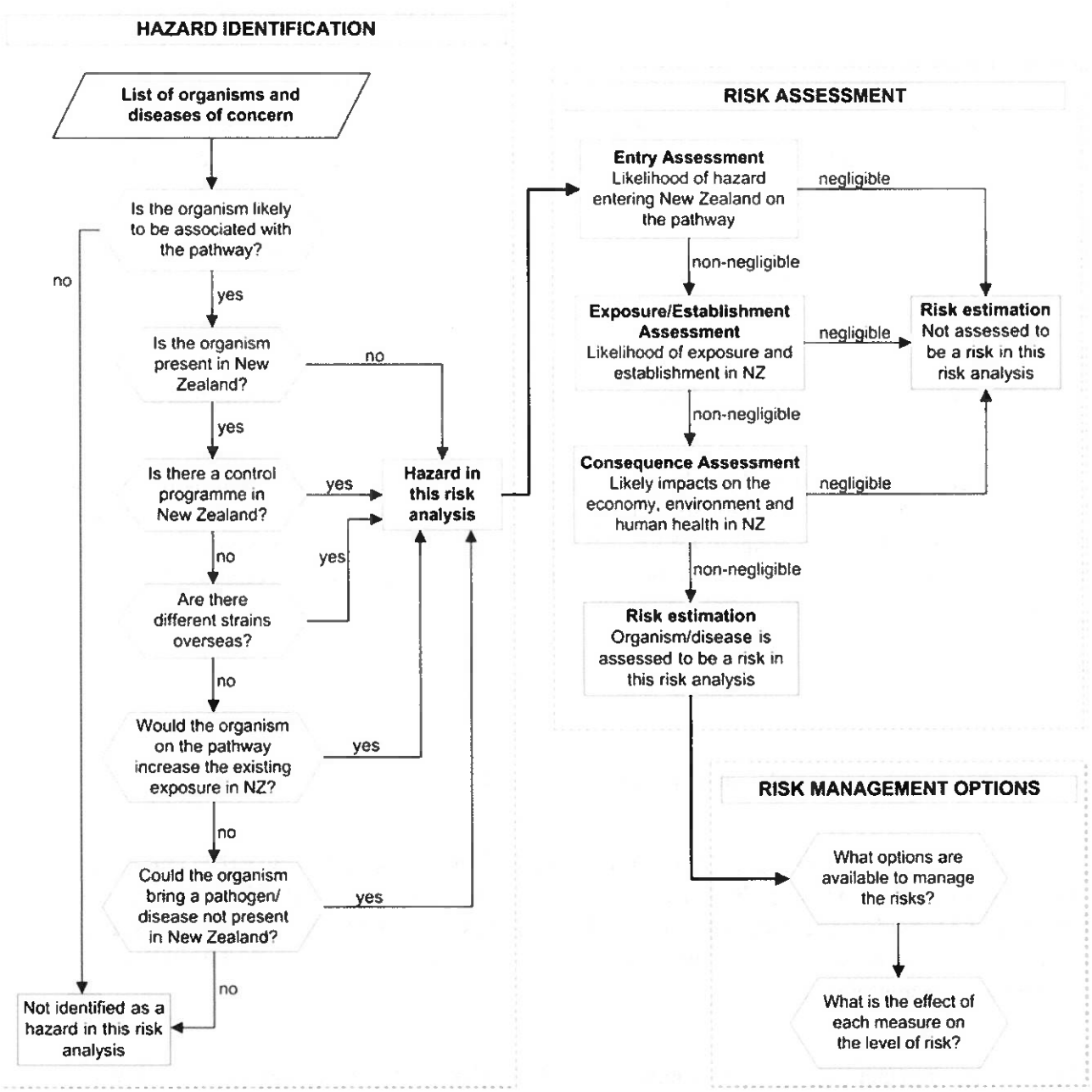
[1] Rapid Risk Assessment: *Mycoplasma bovis* in bovine semen released in September 2017. Updated version released in February 2019.

- Donors tested with an MPI approved test for *M. bovis*
- Semen tested with an MPI approved test for *M. bovis*

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.

Scope and commodity definition

This rapid risk assessment qualitatively assesses the risk due to *M. bovis* associated with the importation of bovine *in-vivo* derived[2] and *in-vitro* produced[3] embryos from approved countries.

Currently *in-vivo* derived embryos are considered to have a very low level of risk for disease transmission, provided strict codes produced by the IETS and OIE are followed. The risk of disease transmission associated with *in-vivo* derived embryos has been categorised by the IETS and is described by the *Code*, Article 4.7.14. Ureaplasma and species of Mycoplasma in cattle are considered by the IETS as Category 4[4] diseases.

It should be noted that categorisation of diseases or pathogenic agents by the IETS, as described for *in-vivo* derived embryos in Article 4.7.14, does not apply in the case of *in-vitro* produced embryos (the *Code* article 4.8.6). Due to different processes of production of *in-vivo* and *in-vitro* embryos, there are differences in the zona pellucida of *in-vivo* derived and *in-vitro* produced embryos. The zona pellucida, an extracellular matrix or shell that surrounds the preovulatory and ovulatory oocyte and early embryos, acts as a barrier against entry and infection of the embryo. However, it is reported that pathogens tend to adhere more firmly to the zona pellucida of *in-vitro* produced embryos, than to that of *in-vivo* derived embryos, making the transmission of pathogens in *in-vitro* embryos a higher probability (Holm & Callesen 1998).

In producing both *in-vivo* and in *in-vitro* bovine embryos it is a prerequisite that:

- Semen used to fertilise oocytes *in-vitro* and *in-vivo* embryos, should meet the health requirements and standards set out in Chapter 4.6. of the *Code* .
- Any biological product of animal origin, including co-culture cells and media constituents, used in embryo collection and oocyte recovery, maturation, fertilisation, culture, washing and storage should be free of living pathogenic agents. Media should be sterilised prior to use by approved methods in accordance with the IETS Manual and handled in such a manner as to ensure that sterility is maintained. Antibiotics should be added to all fluids and media as recommended in the IETS Manual.
- All equipment used to recover, handle, culture, wash, freeze and store oocytes or embryos should be new or cleaned, and sterilised prior to use as recommended in the IETS Manual.

[2] *In-vivo* derived embryos are those recovered after fertilisation and development has occurred in the reproductive tract of the donor female.

[3] *In-vitro* produced embryos are those derived from a process where matured oocytes and capacitated sperm cells are mixed to achieve conception outside the body (i.e. in a laboratory using co-culture cells and fluids) and then cultured to allow development to a morula or blastocyst stage for implantation in a recipient animal.

[4] 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.

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 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).

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).

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[5]). MPI has included sampling and testing of calf rearing properties as part of the phased eradication of *M. bovis* (BNZ 2018).

M. bovis is known to occur worldwide. 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.

In view of these live cattle imports, it is conceivable that *M. bovis* was present in New Zealand prior to this current detection (Nicholas, personal communication[5]). 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[6]). 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[5], Pfutzner & Sachse, 1996).

Epidemiology

The presence of *M. bovis* in the reproductive tract of cows has been reported, both in apparently normal cows (Jain *et. al,* 2012; Langford, 1975) and those with reproductive problems (Stipkovits, 1996; Pfutzner & Sachse 1996). Subclinically infected carrier animals exist and are the most common way that *M. bovis* is introduced into herds (Hazelton *et. al,* 2018).

Following experimental infection by uterine inoculation, the agent has been associated with genital lesions including endometritis, salpingitis and salpingoperitonitis (Hartman, 1964) and placentitis, fetal deaths and abortions (Stallheim and Proctor, 1976). More recently a study by Guo *et. al*, (2014) found that intrauterine infusion with *M. bovis* triggered an endometrial inflammatory response and increased inflammatory cytokines. However, these are experimental studies and the correlation between the artificial dose of *M. bovis* used in these

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

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

studies and the level of *M. bovis* likely to be present in naturally infected cows is unknown, but it is evident that *M. bovis* can be pathogenic to the reproductive tract of cattle.

In field studies Byrne *et. al,* (1999) and Hermeyer *et. al,* (2012) have both isolated *M. bovis* from an aborted bovine fetus.

The prevalence with which the agent occurs in the reproductive tract of cows is unknown. However, based on a study by Langford (1975) which examined 1,265 cervical mucus samples from 192 herds for the presence of mycoplasmas by culture and identified *M. bovigenitalium* in 11% and *M. bovis* in 0.08% of samples, it appears that *M. bovigenitalium* was more commonly detected in the reproductive tract than *M. bovis* in this and other studies (Trichard and Jacobsz, 1985; Kirkbride, 1987). However, due to the variable sensitivities of diagnostic tests in identifying mycoplasmas, the prevalence may have been underestimated (Behera *et. al,* 2018).

In recent years, *M. bovis* has been detected in cattle in many countries and is increasingly recognised as a major pathogen (Wawegama & Browning 2017). It is found worldwide and has spread into new areas, including most European countries and parts of South America, in the last decade (Nicholas & Ayling, 2003).

The mode of infection of the reproductive tract with *M. bovis* remains uncertain. It is possible that haematogenous spread from the udder or lungs may occur, or alternatively that infection of the reproductive tract occurs as a result of breeding or environmental contamination.

Isolations of *M. bovis* from the reproductive tract have been limited to detections in cervico-vaginal mucus (Jain *et. al*, 2012; Feenstra, 1991: Langford, 1975) and uterine fluid (Stipkovits, 1996). It is unknown if *M. bovis* occurs in association with ovarian structures such as oocytes, follicular fluid and cumulus oocyte complexes.

The presence of *M. bovis* in the reproductive tract provides the potential for contamination of embryos or oocytes collected from infected donors.

Both *in-vivo* derived and *in-vitro* produced embryos may retain viable *M. bovis* despite washing in accordance with IETS protocols and standard antibiotic treatments.

The ability of *M. bovis* to remain associated with the surface of zona pellucida-intact embryos and withstand washing has been demonstrated. In general it would appear that such interaction occurs in the same manner in both *in-vivo* derived and *in-vitro* produced embryos. Bielanski *et. al,* (2000) suggested that specific characteristics of mycoplasmas such as the lack of a cell wall, a small diameter and the presence of cytoplasmic projections facilitate a close association with host cells, and consequently make detachment of the agent from the intact zona pellucida of bovine embryos a particular challenge.

Several experimental studies have assessed the effectiveness of various processing methods in eliminating *M. bovis* from contaminated embryos.

In an experimental study by Bielanski *et. al*, (2000), semen contaminated with *M. bovis* at both high and low concentrations was used for oocyte insemination, and the resulting embryos were washed as per IETS recommendations. The authors reported the isolation of *M. bovis* from all of the embryos produced using the semen contaminated with high concentrations and from 60% of embryos produced using the semen contaminated with low concentrations of *M. bovis,* and concluded that supplementation of culture media with

standard antibiotics and multiple washings as per IETS recommendations were ineffective in rendering *in-vitro* produced embryos free of *M. bovis.*

In a study by Riddell *et. al,* (1989), *in-vivo* derived zona pellucida-intact embryos were exposed *in-vitro* to concentrations of *M. bovis* ranging from 1.9×10^6 - 2.9×10^8 cfu/ml. Following exposure to *M. bovis,* the embryos were subjected to various treatments including washing, antibiotic treatment (penicillin 100µg/ml, streptomycin 100µg/ml, amphotericin B 0.25µg/ml for 4 hours at 37 degrees Celsius) and trypsin treatment. *M. bovis* was isolated from all groups of embryos i.e. non-treated (washed only), antibiotic treated and trypsin treated. The authors concluded that recommended procedures such as those outlined by the IETS are ineffective in removing *M. bovis.*

A similar study by Bielanski *et. al,* (1989) exposed *in-vivo* derived embryos to *M. bovis* at concentrations of 1×10^4 - 1×10^6 cfu/ml. Following washing, antibiotic treatment and trypsin treatment of respective groups, *M. bovis* was successfully isolated from all embryos. Again the authors concluded that neither washing nor trypsin treatment nor exposure to combinations of penicillin 2000 IU, streptomycin 2000 IU, lincomycin 600 µg and spectinomycin 1200 µg, or gentamicin 1000 µg, tylosin 200 µg, lincomycin 600 µg and spectinomycin 1200 µg, was effective in removing or inactivating *M. bovis* from embryos.

A study by Riddell *et. al,* (1993b) investigated the effectiveness of selected antimicrobials in inactivating *M. bovis* following *in-vitro* exposure of *in-vivo* derived embryos. Following exposure to *M. bovis*, 10^7 CFU/ml, embryos were treated with kanamycin (200µg/ml), tetracycline (10µg/ml), tylosin (200µg/ml) or a synthesised halamine (5ppm) and subsequently cultured to isolate *Mycoplasma* spp. It was observed that following incubation with the respective antibiotics for a period of 4 hours at 37 degrees Celsius, tylosin was 100% effective for ensuring freedom from *M. bovis*, halamine and kanamycin were 39% and 33% effective, respectively, whilst tetracycline was totally ineffective (Riddell *et. al,* 1993b).

In a similar study by Riddell *et. al,* (1993a), the concentration of kanamycin was increased to 1000 µg/ml and was demonstrated to be completely effective in the inactivation of *M. bovis* whilst producing no apparent detrimental effects on the embryos. In summary Riddell *et. al,* (1993a,b), demonstrated that *in-vivo* derived bovine embryos exposed to *M. bovis* could be effectively treated by supplementation of embryo culture media with 1000µg/ml of kanamycin or 200µg/ml of tylosin and incubation for 4 hours.

Bielanski (2007), in referring to the work of Bielanski (1989) and Riddell (1993a), concluded that effective antimycoplasmic treatment of embryos requires a long exposure to high concentrations of antibiotics. The author concluded that although treatment for 4 hours with tylosin (200µg/ml) or kanamycin (1000µg/ml) was effective in disinfecting bovine embryos following *in-vitro* exposure to *M. bovis*, exposing embryos for only 10 minutes and to lower concentrations of these antibiotics was not effective for the removal of *M. bovis*.

Currently, sanitary control measures and antimicrobial treatment are the only approaches that can be used in attempts to control *M. bovis* infections in herds (Lysnyansky & Ayling, 2016). A review of trends by Lysnyansky & Ayling (2016) conclude that there is increasing evidence of *M. bovis* antimicrobial resistance. These have been based on minimum inhibitory concentration (MIC) levels and genetic analysis and reports increasing resistance of *M. bovis* to the tetracyclines, macrolides, lincosamides, aminoglycosides, chloramphenicols, and fluoroquinolones. Thus certain antimicrobial treatment methods used to disinfect *M. bovis* infected embryos may be ineffective.

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, presently, information relating to the analytical and diagnostic performance of such tests is incomplete, therefore the validity may need to be established.

In summary, *M. bovis* has been isolated, infrequently, from the reproductive tract of cows. Such isolations have occurred both in association with reproductive disease and in clinically normal animals. Specific metabolic and morphological characteristics of mycoplasmas such as the lack of cell wall, a small diameter and the presence of cytoplasmic projections, facilitate a close association with host cells, and consequently make detachment of the agent from the intact zona pellucida of bovine embryos a particular challenge. In addition, mycoplasmas are intrinsically resistant to antimicrobials that interfere with synthesis of folic acid or that act on the cell wall, providing further challenge to the elimination of the agent from the commodity. However, in limited trials, it was observed that following incubation with the respective antibiotics for a period of 4 hours at 37 degrees Celsius, tylosin was 100% effective for ensuring freedom from *M. bovis*, halamine and kanamycin were 39% and 33% effective, respectively (Riddell *et. al,* 1993b). Bielanski (2007) has shown exposing embryos for only 10 minutes and to lower concentrations of these antibiotics was not effective for the removal of *M. bovis*.

Hazard identification conclusion

M. bovis can be present in the reproductive tract of cows and therefore can contaminate oocytes and embryos.

It is concluded that *M. bovis* is identified as a hazard in *in-vivo* derived and *in-vitro* produced embryos.

RISK ASSESSMENT

Entry assessment

The presence of *M. bovis* has been identified infrequently in the reproductive tract of both clinically normal cows (Jain *et. al*, 2012; Langford, 1975) and in cows demonstrating reproductive disorders (Stipkovits, 1996). *Mycoplasma bovis* has been isolated from cervico-vaginal mucus (Jain *et. al,* 2012; Langford 1975) and from uteri (Stipkovits, 1996). However, due to the variable sensitivities of diagnostic tests in identifying mycoplasmas, the prevalence could have been underestimated (Behera *et. al,* 2018).

Experimental studies have demonstrated that both *in-vivo* derived and *in-vitro* produced embryos may retain *M. bovis* infectivity despite standard processing procedures including washing of embryos in accordance with IETS protocols, trypsin treatment and exposure to antibiotic combinations.

Accordingly, the likelihood of entry is assessed to be very low but non-negligible.

Exposure assessment

If we extrapolate the scenario seen in Finland where results strongly support the finding that semen positive for *M. bovis* used in insemination was the source of the *M. bovis* infection (Haapala *et. al,* 2018), and imported embryos are directly implanted into the recipient, this is a highly likely exposure pathway. However, there is no information available on what might

constitute an infectious dose in an embryo/oocyte. It is noteworthy that a larger quantity of semen is used for insemination in comparison to embryos.

The likelihood of transmission of Mycoplasma species associated with *in-vivo* derived embryos has been categorised by the IETS as Category 4. Similar to *in-vitro* produced embryos, the actual likelihood of transmission is unknown.

The presence of naturally-occurring *M. bovis* in the reproductive tract of cows suggests that the multiplication of *M. bovis* in the female reproductive tract can occur. It is feasible that infection via embryo-associated *M. bovis* could result in multiplication within the female reproductive tract followed by spread from initially infected cows to herd mates.

An experimental study by Pavao *et. al,* (2010) observed *in-vitro* produced embryos following exposure to *M. bovis* and reported degeneration and disruption of the zona pellucida of contaminated embryos. Further work in this area is required to determine the effects of *M. bovis* on developing embryos.

In summary, although limited, there is experimental evidence demonstrating the infectivity of *M. bovis* for the reproductive tract. There is a feasible but unproven method of transmission by embryo-associated *M. bovis*. (There are no reports in the published literature of naturally occurring transmission of *M. bovis* from an infected embryo to a recipient dam).

The likelihood of *M. bovis* being transmitted to a recipient via an embryo is unknown. 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.

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[7]). 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.

Therefore, the overall consequence assessment has been assessed as moderate.

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

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 bovine *in-vivo* derived and *in-vitro* produced embryos.

RISK MANAGEMENT

The following information was taken into account when describing options for managing the risks:

- *M. bovis* is not an OIE listed disease and there is no *Code* chapter relating to *M. bovis.*
- *M. bovis* has been isolated in the reproductive tract of cows.
- Experimentally *M. bovis* has been demonstrated to be infective for the bovine female reproductive tract.
- *In-vivo* derived and *in-vitro* produced embryos may retain viable *M. bovis* despite standard processing procedures including washing of embryos in accordance with IETS protocols, trypsin treatment and exposure to antibiotic combinations.
- Internationally traded embryos have not been demonstrated as a transmission pathway for *M. bovis.*
- *M. bovis* is now confirmed in New Zealand following a clinical outbreak in the Canterbury region and has been found in both the North and South Islands.
- The current disease management strategy in New Zealand is to attempt to eradicate.
- Presently, there are no prescribed tests for *M. bovis* for international trade. Current detection methods include culture, molecular and serological detection (Wawegama & Browning, 2017) on milk, joint fluid, bronchiolar lavages, swabs (from different anatomical sites), serum samples (Calcutt *et. al,* 2018), semen or embryos (Bielanski *et. al,* 2000).

Options

Option One

1. Embryos from donor cows are collected, handled, prepared, processed and stored in accordance with chapters 4.7 and 4.8 of the OIE Code.

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

Option Two

1. Embryos from donor cows are collected, handled, prepared, processed and stored in accordance with chapters 4.7 and 4.8 of the OIE Code, and
2. 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 embryos and further transmission over and above that achieved by the OIE Code recommendations alone.

Option Three

1. Embryos from donor cows are collected, handled, prepared, processed and stored in accordance with chapters 4.7 and 4.8 of the OIE Code, and
2. Testing of embryo donors using an MPI approved test.

This option may further reduce the probability of infected embryos and transmission over and above that achieved by the OIE Code recommendations 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[8]), and testing of individual animals rather than the herd is problematic since not all infected animals will develop detectable antibody titres.

Option Four

1. Embryos from donor cows are collected, handled, prepared, processed and stored in accordance with chapters 4.7 and 4.8 of the OIE Code, and
2. A pooled sample of embryos/oocytes, collection fluids and/or washing fluids from each embryo collection has been tested for *M. bovis* using an MPI approved method.

This option also may further reduce the probability of infected embryos and subsequent transmission over and above that achieved by the OIE Code recommendations alone.

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

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.

Given that the level of further reduction in likelihood of entry when any of these tests are applied, they are likely to provide more confidence that the risks are managed to an acceptable level.

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.

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