Ministry for Primary Industries
Manatū Ahu Matua

Risk Management Proposal

Bovine Germplasm RMP draft for consultation

BOVIGERM.GEN

[Document Date]

Growing and Protecting New Zealand

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1 Purpose

The purpose of this document is to:

- Show how options for the management of risk organisms have been considered.
- Provide recommendations for import requirements.

2 Background

Bovine semen and embryos have the potential to harbour exotic viral and bacterial diseases. In February 2009, the Ministry of Agriculture and Forestry (now the Ministry for Primary Industries, (MPI) completed the *Import Risk Analysis (IRA) Cattle Germplasm from All Countries*. This import risk analysis (2009 IRA) forms the basis of the risk management measures in the proposed import health standards (IHSs) for bovine semen and embryos.

Following on from that IRA, the *Import Health Standard: Bovine Semen, BOVSEMID.GE)* and *Import Health Standard: Bovine Embryos, BOVEMID.GE)* were published on 27 June 2011.

These IHSs contain generic import requirements which manage the biosecurity risk associated with importing bovine semen and *in vivo* derived bovine embryos from MPI approved countries.

The generic IHSs served as the basis for country to country (bilateral) negotiations of country-specific veterinary certificates. MPI agreed country specific veterinary certificates with the exporting country's Competent Authority once MPI was satisfied with the exporting country's export systems. Negotiations took into account the verifiable health status of the exporting country, the national systems, legislation and processes in the exporting country for regulatory oversight of the bovine germplasm industry, and the capabilities and preferences of the exporting country's Competent Authority. The assessments were based on the World Organisation for Animal Health OIE Code (the OIE *Code*) section 3, *Quality of Veterinary Services*.

Bovine semen country-specific veterinary certificates are currently available for Australia, USA, Canada, European Union, Norway and Switzerland. Bovine embryo country specific veterinary certificates are currently available for Australia, USA, Canada, the European Union and Switzerland.

Where the IHS requires that the OIE *Code* recommendations are met, the requirements reflect the current OIE *Code* as of 2018. When OIE *Code* chapters are amended, MPI reviews these changes to ensure they continue to align with New Zealand's appropriate level of protection. Where OIE *Code* recommendations no longer meet New Zealand's appropriate level of protection, they are replaced with risk-based MPI recommendations and the IHSs will be amended. Otherwise the most recent version of the OIE *Code* is referred to.

Since the first IRA in 2009, new scientific information and changes to New Zealand's disease status have prompted new risk assessments. The outcomes of those assessments are discussed in this RMP for incorporation into the new IHS. Those assessments include.

- *Rapid Risk Assessment: Mycoplasma bovis in bovine semen in 2019* (RRA bovine semen 2019).
- *Rapid Risk Assessment: Mycoplasma bovis in bovine in-vivo and in-vitro produced embryos* (RRA bovine embryos 2018).
- *Import Risk Analysis: Bovine Leukaemia Virus and Campylobacter fetus* Subspecies *venerealis in Bovine Frozen Semen, In-vivo and In-Vitro Produced Embryos* (IRA BLV and *C. fetus* 2018).
- *Rapid risk assessment: Vesicular stomatitis virus in live animals and their germplasm.*

Additionally, technical advice was provided from MPI's Risk Analysis team on bovine viral diarrhoea virus (BVDV2), and Crimean Congo haemorrhagic fever (CCHF).

This RMP discusses ways to incorporate the new risk advice into the existing IHS. Due to the ongoing *Mycoplasma bovis* eradication programme, there was a preliminary consultation on the *Discussion Document: Mycoplasma bovis risk management in bovine germplasm* from 23 September to 5 November 2019. This RMP describes all proposed amendments in the IHS, including *M. bovis*.

It is proposed that the existing IHSs are combined into one IHS and the proposed title and short-code are *Semen and In Vivo Embryos from Bovines, BOVIGERM.GEN*. The guidance document (GD) will be updated by MPI prior to issuing the IHS. It provides commodity-specific guidance information including links to country-specific bilaterally-agreed veterinary certification for trade in bovine semen and *in vivo* derived bovine embryos.

3 Objective

The objective is to manage, to an appropriate level of protection, the biosecurity risks for bovine semen and bovine embryos, consistent with New Zealand's domestic legislation and international obligations.

New Zealand has adhered to the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) for over two decades, and in doing so recognises the desirability of using agreed international standards for trade wherever possible. Under Article 2.1 of the World Organisation for Animal Health (OIE) *Code* Chapter on Import Risk Analysis, "The objective is to manage risk appropriately to ensure that a balance is achieved between a country's desire to minimise the likelihood or frequency of disease incursions and their consequences and its desire to import commodities and fulfil its obligation under international trade agreements." And under Article 5.3, "Members may choose to implement sanitary measures more stringent than those in international standards, if these are deemed necessary to protect animal or human health and are scientifically justified by a risk analysis."

Of the hazards identified in the import risk analyses, the 2011 IHS included risk management for the following hazards:

- Bluetongue virus
- Borna disease virus
- Exotic bovine herpes viruses (semen only)
- Bovine viral diarrhoea virus type 2
- Crimean Congo haemorrhagic fever
- Foot and mouth disease virus
- Lumpy skin disease virus
- Rift Valley fever virus
- Vesicular stomatitis virus
- Exotic *Brucella* spp. (semen only)
- *Coxiella burnetii*
- *Mycobacterium bovis*
- *Mycoplasma mycoides* subsp. *mycoides* SC
- Other exotic *Mycoplasma* spp.

Risk management was not considered justified in 2011 for the following hazards identified by the IRA:

- *Campylobacter fetus* subspecies *venerealis* (semen only)
- *Chlamydophila abortus*
- Bovine leukaemia virus (semen only)
- Exotic *Salmonella* spp.
- Exotic *Leptospira* spp.

Identified hazards proposed for addition to the IHS in the 2020 amendment include:

- Bovine leukaemia virus
- *Campylobacter fetus* subspecies *venerealis*

Identified hazards proposed for removal in the 2020 amendment include:

- Bluetongue virus
- Borna disease virus
- Crimean Congo haemorrhagic fever
- Vesicular stomatitis virus

4 General requirements for all importations of bovine germplasm

The general requirements section of the IHSs include risk management measures for semen and embryo donors, regardless of the country's disease freedom claims. Where possible, these requirements align with the recommendations of the OIE *Code* and the International Embryo Transfer Society (IETS) Manual.

4.1 Application

(1) The IHS applies to frozen semen and *in vivo* derived embryos from the Bovinae subfamily for import from all countries into New Zealand.

4.2 Diagnostic tests, vaccines and treatments

(1) All pre-export and/or surveillance testing required by the IHS must be:

a) conducted by a laboratory approved by the Competent Authority of the exporting country; or
b) conducted by a laboratory approved by the Competent Authority of any other country approved to export the specified type of bovine germplasm to New Zealand.

(2) All laboratory samples required by the IHS must be collected, processed, and stored in accordance with the recommendations in the OIE *Code* and/or the *Manual* or as described in the document, *Approved Diagnostic Tests, Vaccines, Treatments and Post-arrival Testing Laboratories for Animal Import Health Standards, MPI-STD-TVTL.*

(3) All diagnostic test(s) and vaccines that are required to be used or undertaken by the IHS must be those that have been approved by MPI for that purpose and documented in *MPI-STD-TVTL.* MPI's approval process includes consultation with the MPI Animal Health Laboratory (AHL) and the test must be deemed valid for diagnostic purposes in bovines and must be appropriate for surveillance for the identified risk organism.

(4) All products and vaccinations required by the IHS to be administered to meet the specific disease requirements in Part 2 of the IHS must have been administered according to the manufacturer's instruction in a country that the CTO has agreed meets the requirements of clause 1.5. All vaccinations must be either the final dose of a primary vaccination course or the recommended booster to complement the primary course.

(5) Where products required by the IHS have been administered, the product name, manufacturer, active ingredients (where applicable), and the dose and date of the treatment must be recorded on the veterinary certificate.

(6) Where vaccines required by the IHS have been administered, all vaccine names, whether they are inactivated or modified live virus, and the virus types and strains included in the vaccine must be recorded on the veterinary certificate.

4.3 Semen collection centre requirements

(1) Semen collection must be carried out in a semen collection centre that complies with the recommendations for centres in the *Code* chapter *General Hygiene in Semen Collection and Processing Centres.*

(2) The semen collection centre must be:

a) approved for export by the Competent Authority;
b) subjected to regular inspection, at least every 12 months, by an Official Veterinarian; and
c) under the supervision of a semen collection centre veterinarian approved by the Competent Authority.

(3) The name and approval number of the semen collection centre must be recorded on the veterinary certificate.

(4) Semen donors may be transferred from one approved semen collection centre to another approved centre of equal health status without isolation or testing if the Competent Authority ensures that all of the following requirements are met:

a) Donors have been examined by the approved semen collection centre veterinarian and show no evidence of infectious disease transmissible in semen on the day of entry into the centre.
b) Transfer is direct.
c) Donors are protected from insect attack during transit.
d) Donors do not come into direct or indirect contact with animals of lower health status.
e) The means of transport is disinfected before use.

4.4 Donor requirements

(1) Semen donors must meet the requirements in the *Code* chapter *Collection and Processing of Bovine, Small Ruminant, and Porcine Semen*, and any additional requirements in *Part 2: Specified Requirements for Identified Risk Organisms* of this IHS.

(2) During the 28 days in which semen donors are held in pre-entry isolation prior to entering the semen collection centre (as prescribed in the OIE *Code*), they must not be used for natural mating, and must be isolated from animals not of equivalent health status.

(3) Embryo donors must meet the recommendations in the *Code* chapter *Collection and Processing of In Vivo Derived Embryos from Livestock and Equids* and any additional requirements in *Part 2: Specified Requirements for Identified Risk Organisms* of this IHS.

(4) Embryo donors must be resident in the embryo collection herd for at least 28 days prior to embryo collection for export to New Zealand. While resident with the collection herd, the herd must not be subject to veterinary restrictions for the identified risk organisms managed in Part 2 of the IHS.

(5) During the 28 days in which embryo donors are resident with the embryo collection herd, they must be isolated from animals not of equivalent health status.

(6) Donors that were imported to the exporting country must have lived continuously in an approved country for at least 60 days before germplasm collection.

(7) On the day of germplasm collection, the embryo collection team veterinarian or semen collection centre veterinarian must determine that the donor is free from clinical evidence of infectious diseases transmissible in germplasm.

(8) Where a specific requirement of the IHS for a risk organism is met by pre-collection testing, the germplasm donors must be isolated from other animals not of equivalent tested health status, from the time of the pre-collection test until completion of collection for export.

(9) Where a specific requirement of the IHS for a risk organism is met by monitoring the germplasm donor for clinical signs for a specified time after collection, the germplasm must be stored for that amount of time prior to export.

4.5 Semen collection, processing and storage

(1) Semen collection, processing and storage must comply with the sections relevant for bovine semen in the *Code* chapter *Collection and Processing of Bovine, Small Ruminant, and Porcine Semen*.

(2) Where Part 2 requires testing within a certain time period before or after semen collection:

a) Semen collection may be a time period of up to 60 consecutive days.
b) Samples for testing before collection must be obtained within the specified period before the first day of the semen collection period.
c) Tests required after semen collection must have samples collected within the specified period after the last day of the semen collection period.

(3) A cryogenic or cooling agent used in the freezing process, storage and transport must not have been used previously in association with any other product of animal origin.

(4) All straws must be sealed, and clearly and permanently marked to identify the donor and the date(s) of freezing. The markings must conform to international standards of the *International Committee for Animal Recording (ICAR)*. If a code is used for this information, its decipher instructions must accompany the consignment.

(5) Semen may only be stored with germplasm that has been collected and processed in accordance with the *Code*.

(6) Semen must be held in a storage place approved by the Competent Authority of the exporting country until the time of export.

(7) Subject to clause 8, semen may only be imported into New Zealand if the semen is imported directly from the country in which it was collected.

(8) If semen is collected in a country that meets the requirements of clause 1.5 of the IHS and stored in another country (exporting country) that meets the requirements of clause 1.5 of the IHS, that semen may be imported into New Zealand if the consignment is accompanied by:

a) A declaration from the Competent Authority of the exporting country identifying the semen from the origin country as the semen being exported to New Zealand.
b) A veterinary certificate from the Competent Authority of the exporting country that certifies that the semen has been stored and transported in the exporting country in accordance with the requirements of this IHS.
c) Evidence that the semen was collected, processed, and stored in the origin country in accordance with the requirements of this IHS in the form of either:

i) A veterinary certificate issued by the Competent Authority of the origin country certifying that the semen meets the requirements of this IHS; or
ii) A letter from the Competent Authority of the origin country confirming the semen meets the requirements of this IHS.

4.6 Embryo collection, processing and storage

(1) Embryos must be collected, washed, processed, stored and traceability maintained under the supervision of an embryo collection team veterinarian and in accordance with the recommendations in the OIE *Code* chapters on *Collection and Processing of In Vivo Derived Embryos from Livestock and Equids.*

(2) The embryo collection team must operate in accordance with the conditions listed in the OIE *Code* chapters on *Collection and Processing of In Vivo Derived Embryos from Livestock and Equids.*

(3) Embryos must be collected, washed, processed, stored and traceability maintained under conditions that comply with the recommendations in the IETS *Manual*.

(4) At the time of embryo collection each embryo must be examined over its entire surface at not less than 50X magnification and found to have an intact *zona pelludica* and be free of adherent material.

(5) Any micro-manipulation that causes a breach of the *zona pellucida* must be done as per the procedures described in the OIE *Code* chapter *Collection and Processing of Micromanipulated Oocytes or Embryos from Livestock and Horses* and the IETS *Manual*.

a) These include specifications on the facilities used and require that micro-manipulation only be carried out on an embryo having an intact *zona pellucida* and that it be done subsequent to the last wash and examination of the embryo.

(6) All biological products of animal origin used in the media and solutions for collection, processing, washing or storage of embryos must be free of pathogenic organisms including pestiviruses and prions. Media and solutions must be sterilised by approved methods according to the IETS *Manual* and handled in such a manner as to ensure that sterility was maintained.

(7) All straws must be sealed, and clearly and permanently marked to identify the donor and the date(s) of freezing. The markings must conform to international standards of the *International Committee for Animal Recording* (ICAR; www.icar.org) and the IETS *Manual*. If a code is used for this information, its decipher instructions must accompany the consignment.

(8) Embryos may only be stored with germplasm that has been collected and processed in accordance with the OIE *Code*.

(9) Embryos must only be held in a storage place approved by the Competent Authority of the exporting country until the time of export.

(10) Subject to (11), embryos can only be imported into New Zealand if the embryos are imported directly from the country in which they were collected.

(11) If embryos are collected in a country that meets the requirements of clause 1.5 and stored in another approved country (exporting country), those embryos may be imported into New Zealand if the consignment is accompanied by:

a) A declaration from the Competent Authority of the exporting country identifying the embryos from the origin country as the embryos being exported to New Zealand;
b) A veterinary certificate from the Competent Authority of the exporting country that certifies that the embryos have been stored and transported in the exporting country in accordance with the requirements of this IHS;
c) Evidence that the embryos were collected, processed, and stored in the origin country in accordance with the requirements of this IHS in the form of either:

i) A veterinary certificate issued by the Competent Authority of the origin country certifying that the embryos meet the requirements of this IHS; or
ii) A letter from the Competent Authority of the origin country confirming how the embryos meet the requirements of this IHS.

4.7 Transport

(1) All transport containers for transport to New Zealand must be new or disinfected, and confirmed empty prior to loading. When a transport container is disinfected, the disinfectant, its active chemical and the date of disinfection must be recorded on the veterinary certificate.

(2) The container must only be filled with fresh (previously unused) liquid nitrogen.

(3) All transport containers in which germplasm is transported to New Zealand must be sealed, by either the collection centre veterinarian or an Official Veterinarian, using tamper-evident seals that are positioned to ensure that no germplasm can be added after the tank has been sealed. The seal number must be recorded on the veterinary certificate.

(4) Where germplasm is transferred from one transport container to another, the date of transfer, approved collection centre, reason for transfer, and the name of veterinarian involved in the transfer must be recorded on the veterinary certificate.

5 Recommendations for identified risk organisms

5.1 Bluetongue (BT)

5.1.1 Risk management options presented in the 2009 IRA

The 2009 IRA assessed the risk of bluetongue virus for bovine germplasm to be negligible.

5.1.2 Discussion

(1) Measures have been maintained to align with international recommendations. However measures are no longer considered justified. Several factors led to this conclusion:

a) New Zealand is free of *Culicoides* vectors, the only known vectors capable of transmitting BTV.
b) New Zealand monitors for *Culicoides*, including monitoring sentinel cattle for seroconversion to arboviruses.
c) According to the OIE *Code*, New Zealand will not lose its bluetongue free status through the importation of vaccinated, seropositive or infective animals, or semen or embryos/ova from infected countries or infected zones.
d) The viraemic period in cattle can be up to 11 weeks. However cattle are not carriers.
e) Bluetongue is not a zoonotic disease

5.1.3 Recommendation

(1) Measures are not justified.

5.2 Borna disease

5.2.1 Risk management options presented in the 2009 IRA

(1) Require germplasm donors to have been resident since birth in countries where the disease has never been reported.

(2) Require donors to originate from herds with a greater than 5 year history of freedom from the disease in countries in which the disease is notifiable or in which reliable histories are available.

(3) Test aliquots of semen and embryos from each collection by intracerebral inoculation into rabbits or by culture on cell cultures from embryonic rabbit or rat brain with negative results.

(4) PCR test of peripheral cells from donors with negative results (accuracy and reliability of this testing methodology have been questioned.)

(5) It is proposed that all of the options suggested in the risk analysis be used, in modified form, in the import health standard.

5.2.2 Discussion

(1) Borna disease (BD) is not an OIE listed disease.

(2) There is no evidence that it is venereally transmitted in any species of animal and the public health impact remains only speculative. Measures were excluded from the IHSs for ovine and equine germplasm, and those are the species primarily affected by the disease.

5.2.3 Recommendation

(1) Measures are not justified.

5.3 Bovine herpes virus 1.1, 1.2a, and 5 (Infectious Bovine Rhinotracheitis/Infectious Pustular Vulvovaginitis, IBR/IPV)

5.3.1 Risk management options presented in the 2009 IRA

(1) Test each batch of semen by a VI test (or a suitable PCR test when available).

(2) Require that donors be from herds or breeding centres that are maintained free from IBR in accordance with the OIE *Code*.

(3) Require isolation of donor animals for at least 30 days after semen collection and testing by a validated serological test with negative result, at least 21 days after semen collection.

5.3.2 Discussion

(1) Abortions and encephalitis have not been associated with BHV in New Zealand, where only BHV1.2b has been isolated. Both syndromes of BHV infection, IBR and IPV, are present in New Zealand.

(2) Chronically infected latent carriers exist and are known to excrete BHV in their semen.

(3) A 2005 study demonstrated that seronegative breeding bulls can produce BHV infected semen. There appears to be little other research that confirms this finding. It is generally agreed that the OIE *Code* recommendations appropriately manage the risk in bovine semen originating from AI centres that meet the OIE *Code's* hygiene, collection, and processing recommendations.

(4) In 2010, there were multiple submissions requesting that MPI align with the OIE, by not requiring post-collection serological testing. It was understood that there are quality controls in place that help remove BHV infected semen from international trade and that a pre-collection testing appropriately manages the risk.

(5) After decades of importing under the existing measures, the IHS should continue to align with the OIE *Code*. Aligning with the *Code* requires that the 30 day post-collection isolation period is added to the IHS's post-collection test requirement.

(6) The virus is known to adhere to embryos, but it is removed when IETS washing protocol (with trypsin) is followed. It is IETS category 1 disease and embryos should be collected, processed and stored in accordance with the OIE *Code*. No additional donor or germplasm testing is recommended.

5.3.3 Recommendation

(1) At the time of collection of semen for export to New Zealand, the exporting country must be free from BHV 1.1, BHV 1.2a and BHV5 in accordance with the OIE *Code*; or

(2) The semen collection centre must be maintained free from BHV 1.1, 1.2a, and 5 from commencement until conclusion of semen collection for export to New Zealand, through compliance with the recommendations in the OIE *Code* in relation to BHV, including:

a) The centre must:

i) Test all cattle prior to pre-entry isolation for antibodies using a test listed in *MPI-STD-TVTL*, with negative results;
ii) Test all cattle in pre-entry isolation for antibodies, with negative results, or where an animal in a group has tested positive re-testing the remaining animals, with negative results, not less than 21 days after removal of the positive animal; and
iii) Thereafter, annually re-test all donors for antibodies, with negative results; or

(3) The semen donor must be:

a) held in isolation for the 30 days following collection;
b) tested for BHV 1.1, 1.2a, and 5 using a test listed in *MPI-STD-TVTL* at least 21 days after semen collection for export to New Zealand, with negative results; or

(4) An aliquot of semen from each semen collection for export to New Zealand must be tested for BHV1.1, 1.2a, and 5 with a test listed in *MPI-STD-TVTL*, with negative results.

(5) Measures are not justified for embryos.

5.4 Bovine leukaemia virus, BLV (Enzootic Bovine Leukosis, EBL)

5.4.1 Risk management options presented in the 2018 IRA

(1) The semen was collected, processed and stored in accordance with Chapters 4.6 and 4.7 of the OIE *Code*.

Note: This is a general requirement for all imported semen.

(2) The semen was collected, processed and stored in accordance with Chapters 4.6 and 4.7 of the OIE *Code*; and

a) An aliquot of not less than 0.5 ml of processed semen from the final collection of each donor was tested by an approved virus isolation test or a polymerase chain reaction test with negative results.

Note: This option would likely further reduce the probability of infected semen over and above that achieved by Option 1 alone.

(3) The semen was collected, processed and stored in accordance with Chapters 4.6 and 4.7 of the OIE *Code*; and

a) The donor bull was resident at the time of semen collection in an Enzootic Bovine Leukosis (EBL) free herd in accordance with the OIE *Code*.
b) If less than two years of age, the bull came from a serologically negative 'uterine' dam; or

i) The bull was subjected to diagnostic tests for EBL on blood samples on two occasions with negative results, the first test being carried out at least 30 days before and the second test at least 90 days after collection of the semen.

Note: This option aligns with the OIE *Code* recommendation for the importation of bovine semen.

5.4.2 Discussion

(1) The preliminary hazard list compiled in the 2009 IRA considered bovine leukaemia virus as endemic in New Zealand, therefore, no risk management was recommended (2009 IRA).

(2) In 1997 New Zealand dairy industry implemented the "Dairy Enzootic Bovine Leukosis" scheme. Under this scheme the last BLV-infected cows were culled in 2007-2008. Since then there have been no detections of BLV in New Zealand and annual screening of the dairy industry was maintained at over 50% of all herds for several years (Voges, 2012)[1].

(3) BLV, the causative agent of EBL is notifiable under the Biosecurity (Notifiable Organisms) Order 2016.

(4) BLV is subject to passive surveillance in New Zealand.

(5) Transmission of BLV occurs through the transfer of infected lymphocytes and BLV DNA has been isolated in semen.

(6) The likelihood of BLV being present in semen is assessed to be low. The likelihood of subsequent exposure and transmission of BLV to susceptible animals is assessed to be low. The consequences of entry and establishment of BLV are assessed to be non-negligible. Bovine leukaemia virus is therefore assessed to be a risk in imported bovine semen. Risk management is therefore scientifically justified.

(7) The OIE *Code* provides recommendations for the importation of bovine semen.

[1] Voges, (2012). Reports from industry surveillance and disease control programmes: New Zealand Dairy Enzootic Bovine Leukosis (EBL) Control Scheme, Surveillance, 39 (3):41

(8) PCR on semen is considered to be more sensitive than virus isolation.

(9) Lymphocytes may infrequently leak into the genital tract. If only semen were tested, a sample from every collection would need to be tested. Testing diluted semen is considered acceptable due to low risk of BLV being present in semen.

(10) The 2018 IRA concluded that BLV is not a risk in *in vivo* derived bovine embryos. The washing protocols have been shown to be effective to remove embryo-associated BLV (IETS Category 1).

5.4.3 Recommendation

(11) The semen donor must be resident at the time of semen collection in an EBL-free herd in accordance with the OIE *Code*; and

a) if less than two years of age, the semen donor must come from a serologically negative 'uterine' dam; or
b) the semen donor must be subjected to a test listed in *MPI-STD-TVTL* for EBL on blood samples on two occasions with negative results, the first test being carried out at least 30 days before and the second test at least 90 days after collection of the semen; or

(12) An aliquot of semen from each collection for export to New Zealand must be tested for BLV with a test listed in *MPI-STD-TVTL*, with negative results.

(13) Measures are not justified for embryos.

5.5 Bovine viral diarrhoea virus genotype 2 (BVDV2)

5.5.1 Risk management options presented in the 2009 IRA

(1) Require donor bulls to have been resident on a semen collection centre where bulls are maintained and tested as specified in the OIE *Code* chapter on collection and processing of bovine semen. [This includes testing the collection herd prior to pre-entry isolation by virus isolation or virus antigen test and a serological test, then full repeat testing in pre-entry isolation].

(2) Require each batch of semen be tested by VI or reverse transcriptase polymerase chain reaction (RT-PCR).

(3) Require that embryo donors be tested as bulls are in semen collection centres (as per OIE *Code* chapter on collection and processing of bovine semen).

(4) Require non-fertilized, degenerated, and zona pellucida compromised embryos, collection fluid and washing fluid (or an embryo from the first embryo collection for each consignment) from each donor be collected and be tested by VI or RT-PCR, with negative result.

5.5.2 Discussion

(1) BVDV2 has not been described in cattle in New Zealand.

(2) Both acutely infected and persistently infected semen donors need to be prevented from supplying semen for export to New Zealand.

(3) The antigen detection tests are not appropriate for detecting acutely infected cattle due to their transient viraemia. Semen donors should be tested for antibodies at least 3 weeks apart (prior to pre-entry isolation and in pre-entry isolation) to detect acutely infected donors. During isolation, acutely infected cattle would be expected to show a 4-fold rise in antibody titre.

(4) BVDV is an IETS Category 3 disease[2] with regard to the risk of disease transmission via *in vivo* derived embryos. A 2013 study showed that *in vivo* produced embryos infected with BVDV when exposed to BVDV infected semen do not transmit the virus to recipients or offspring when washed with trypsin according to IETS washing requirements. Embryos should be considered lower risk than semen.

(5) A 14 day isolation period for embryo donors should be considered to cover the incubation period (3-7 days) for acute BVD infections. As BVDV-2 can cause severe disease, animals displaying clinical signs will be identified and excluded as embryo donors. Acutely infected animals would be expected to clear the virus prior to collection.

(6) Persistently infected cattle do not have detectable antibodies, therefore the antigen detection ELISA, real-time RT-PCR and virus isolation, as described in the OIE *Manual*, should be required. Semen donors should be tested twice, 3-4 weeks apart.

(7) Embryo donors need only be subjected to a single viral antigen or RNA detection test 28 to 14 days prior to collection in order to be confident that they are not persistently infected. The donor should be isolated from untested cattle between the test date and embryo collection.

(8) If any semen donor is serologically positive prior to entering isolation, the semen must be confirmed negative with the virus isolation or antigen detection ELISA.

(9) Animals which remain in collection centres should be tested annually.

(10) Germplasm can be tested by PCR. Test results from a pooled embryo sample or semen sample from the same day of collection can be considered representative of the collection.

(11) The current measures are appropriate. For clarification, it should be stated that testing should be within the 30 days prior to the start of pre-entry isolation.

5.5.3 Recommendation

(1) At the time of germplasm collection for export to New Zealand, the exporting country must be recognised by the CTO as free from BVDV2; or

(2) Semen:

a) The semen collection centre must be maintained free from BVDV2 from commencement until conclusion of semen collection for export to New Zealand, through compliance with the recommendations in the OIE *Code* in relation to BVDV2, and:

i) The collection centre must:

1) Test all cattle within 30 days prior to pre-entry isolation with a test listed in *MPI-STD-TVTL*;
2) Test all cattle after 21 days isolation with a test listed in *MPI-STD-TVTL*;
3) If any animal seroconverts, keep all animals in pre-entry isolation until there is no more seroconversion for 3 weeks;
4) Only approve entry for groups where pre-entry isolation results indicate the absence of antigen-positive cattle;
5) Thereafter, annually re-test seronegative cattle;
6) For seropositive donors, test semen for BVDV with a test listed in *MPI-STD-TVTL* with negative results, prior to use of that animal as a semen donor; or

b) An aliquot of semen from each semen collection for export to New Zealand must be tested for BVDV2 with a test listed in *MPI-STD-TVTL*, with negative results.

(3) Embryos:

a) The embryo donor must be tested for BVDV2:

[2] According to the IETS Manual, Category 3 diseases or pathogenic agents are those for which preliminary evidence indicates that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer in accordance with the IETS Manual, but for which additional in vitro and in vivo experimental data are required to substantiate the preliminary findings.

i) between 28-14 days prior to collection with a test listed in *MPI-STD-TVTL*;
ii) isolated from untested cattle between the test date and embryo collection; or

b) A pooled sample of embryos/oocytes, collection fluids and/or washing fluids from each embryo collection for export to New Zealand must be tested for BVDV2 with a test listed in *MPI-STD-TVTL*, with negative results.

5.6 Crimean Congo haemorrhagic fever virus (CCHF)

5.6.1 Risk management options presented in the 2009 IRA

(1) Require germplasm donors to have been resident for at least the 21 days before germplasm collection in a country or zone that is free from the disease.

(2) Require scrupulous treatment of germplasm donors with acaricide, and inspection and placement in tick free germplasm collection premises. Donors would be required to be quarantined for at least 3 weeks prior to start off, and during germplasm collection with regular inspection and maintenance of tick free status.

(3) Require serological testing of germplasm donors within 7 days prior to start of germplasm collection and 3-8 weeks after germplasm collection.

5.6.2 Discussion

(1) Presence of the virus or transmission of the virus by insemination or implantation of germplasm (of any species) has never been described.

(2) Hyalomma ticks are considered to be required for the establishment of the disease. There is no evidence that the New Zealand cattle tick is a competent vector.

(3) Although never previously demonstrated, if a recipient dam were to become viraemic with CCHF virus, she would remain so for only up to 7 days. There are no descriptions of long term carriers. During viraemia, her blood would pose a zoonotic risk.

(4) A risk assessment conducted 2016 concluded that ruminant germplasm is not a viable pathway to enter New Zealand.

5.6.3 Recommendation

(1) Measures are not justified.

5.7 Foot and mouth disease virus (FMD)

5.7.1 Risk management options presented in the 2009 IRA

(1) Institute measures in accordance with the OIE *Code* for semen and embryos.

(2) Prohibit importation of germplasm from countries that are infected with foot and mouth disease.

5.7.2 Discussion

(1) Due to the extreme seriousness of the disease and the catastrophic consequences that would result from its introduction, it was concluded that importation of bovine germplasm should be limited to countries or zones that are free of FMD virus in accordance with the OIE *Code*, or countries or zones in which compliance with measures in accordance with the recommendations of the OIE *Code* for import of germplasm from FMD infected countries or zones has been reviewed and accepted by MPI.

(2) The OIE *Code's* recommendation for importing from a FMD free country include monitoring the semen donor for 30 days after collection for clinical signs of FMD. Therefore all semen must be frozen for 30 days before export to New Zealand.

(3) While the OIE *Code* indicates that no additional measures are justified for embryos, the measures recommended for semen from free countries or zones are:

(4) Semen Donors:

a) showed no clinical signs of FMD on the day of collection of the semen and for the following 30 days;
b) were kept in a centre where no animal had been added in the 30 days before collection, and that FMD has not occurred within a 10 kilometre radius of the centre for the 30 days before and after collection; either

i) have been vaccinated at least twice, with the last vaccination not less than one month and not more than six months prior to collection, unless protective immunity has been demonstrated for more than six months; or
ii) were subjected, not less than 21 days after collection of the semen, to tests for antibodies against FMDV, with negative results;

(5) Semen:

a) was collected, processed and stored in accordance with Chapters 4.6 and 4.7 of the *Code*;
b) was subjected, with negative results, to a test for evidence of FMDV if the donor males has been vaccinated within the 12 months prior to collection;
c) was stored in the country of origin for a period of at least one month following collection, and that during this period no animal on the establishment where the donor males were kept showed any sign of FMD.

5.7.3 Recommendation

(1) No change in risk management from the current IHS's FMD requirements.

(2) Semen:

a) The donor must be resident for at least the 3 months before semen collection in a country or zone that is free from FMD without vaccination in accordance with the OIE *Code*; or
b) The herd of origin, semen collection centre, donor animal and semen for export must comply with OIE *Code* recommendations for export of bovine semen from countries or zones presenting a risk of FMD; and

i) Each semen collection, processing and storage facility in the exporting country intended to be used during the preparation of an export consignment to New Zealand must be approved by an MPI Chief Technical Officer (CTO).

(3) Embryos:

a) The donor must be resident for at least the 3 months before embryo collection in a country or zone that is free from FMD without vaccination in accordance with the OIE *Code*; or
b) The herd of origin, embryo collection herd where the donors were resident during embryo collection, donor animal and embryos for export must comply with the OIE *Code* FMD Article *Recommendations for the Importation of In Vivo Derived Embryos of Cattle*; and

i) Each embryo collection, processing and storage facility in the exporting country, intended to be used during the preparation of an export consignment to New Zealand, must be approved by an MPI CTO.

Note: The approval will be dependent on the establishment, its location and operating standards, and that the verification systems of the veterinary authority achieve a very high level of risk management for FMD. The process for MPI approval may include site inspection. MPI reserves the right to supervise collection or require any other measures deemed necessary to ensure compliance with facility and operating standards upon which the approval is based.

5.8 Lumpy skin disease virus (LSD)

5.8.1 Risk management options presented in the 2009 IRA

(1) Donors have been resident for 6 months prior to germplasm collection in a country or zone that is free of LSD as defined by the OIE *Code*.

(2) Donors could be required to be resident in an establishment or germplasm collection centre that has been free from LSD for at least 6 months. All animals on the centre could be required to be free from any sign of LSD for at least 28 days after completion of germplasm collection.

(3) Aliquots of semen and embryo wash fluid, substandard embryos or aliquot of embryos, from each batch of imported germplasm could be tested for LSD with a test listed in *MPI-STD-TVTL.*

5.8.2 Discussion

(1) Each of the options presented in the import risk analysis were included, in modified form, in the recommended options for the IHS.

5.8.3 Recommendation

(1) No change from the current IHS LSD requirements.

(2) The germplasm donor must be resident for 6 months prior to germplasm collection in a country or zone that is free of LSD as defined by the OIE *Code*; or

(3) The germplasm donor must be resident in an establishment that was free of clinical evidence of LSD during a period from at least 6 months prior to commencement, until 28 days after conclusion of germplasm collection for export to New Zealand; or

(4) An aliquot of semen or a sample of embryos/oocytes, collection fluids and/or washing fluids from each germplasm collection for export to New Zealand must be tested for LSD with a test listed in *MPI-STD-TVTL* with negative results.

5.9 Rift Valley fever virus (RVF)

5.9.1 Risk management options presented in the 2009 IRA

(1) Donors have been resident for 30 days prior to collection of germplasm, and during germplasm collection, in a RVF free country or zone.

(2) Donors have been resident for 6 months prior to and during collection of germplasm in a RVF infected country in which climatic changes predisposing to RVF outbreaks have not occurred in the previous 6 months.

(3) Donors were held in mosquito-free premises at least 30 days prior to, and during collection.

5.9.2 Discussion

(1) The country freedom option presented in the import risk analysis was included in the IHS. The other options in the IHS were worded to align more closely with the OIE *Code*. The *Code* RVF chapter has been amended since 2011 and the new recommendations should be adopted. They now allow for donors to be seropositive or vaccinated. The risk is considered to be managed since long term carriers of the virus have not been described. Vaccination would need to be in accordance with the OIE.

5.9.3 Recommendation

(1) The donor must be resident, for at least the 30 days prior to, and during germplasm collection for export to New Zealand in a country or zone that is free from RVF in accordance with the OIE *Code*; or

(2) The donor showed no sign of RVF within the period from 14 days prior to and 14 days following germplasm collection; and either

a) the donor must be vaccinated against RVF at least 14 days prior to collection; or
b) the donor must be demonstrated to be seropositive on the day of collection with a test listed in *MPI-STD-TVTL*; or
c) testing of paired samples with a test listed in *MPI-STD-TVTL* must demonstrate that seroconversion did not occur between germplasm collection and 14 days after.

5.10 Vesicular stomatitis virus (VS)

5.10.1 Risk estimation presented in the 2016 rapid risk assessment:

(1) For animal germplasm, the likelihood of entry is assessed to be negligible. Accordingly, the risk estimate for animal germplasm is negligible.

5.10.2 Discussion

(1) OIE has removed VS from its notifiable disease list.

(2) There is a considerable body of evidence showing that viraemia does not occur in VS, introduction via germplasm is therefore not considered possible.

5.10.3 Recommendation

(1) It is recommended the measures for VS are removed in alignment with the OIE *Code*. No specific measures are necessary.

5.11 *Brucella melitensis, Brucella suis, and Brucella abortus* (bovine brucellosis)

5.11.1 Risk management options presented in the 2009 IRA ~~for bovine germplasm~~

(1) Donor bulls could be required to be kept since birth in a country or zone that is officially free from brucellosis.

(2) Donor bulls could be housed at an artificial breeding centre where the testing programme for bulls includes testing with both the complement fixation test and the buffered antigen agglutination test.

(3) Donor bulls could be required to be kept in a herd officially free from bovine brucellosis, showed no clinical sign of bovine brucellosis on the day of collection of the semen and were subjected to a buffered Brucella antigen test with negative results during the 30 days prior to collection. (*Note*: This option is for bulls not maintained on an approved semen collection centre. The standard will require that bulls are housed on a semen collection centre.)

(4) Donor bulls were kept in a herd free from bovine brucellosis, showed no clinical sign of bovine brucellosis on the day of collection and were subjected to the buffered Brucella antigen and complement fixation tests with negative results during the 30 days prior to collection. (*Note*: This option is for bulls not maintained on an approved semen collection centre.)

(5) Each of the options suggested in the import risk analysis were included as recommended options in the import health standard in modified form.

5.11.2 Discussion

(1) *Brucella abortus, Brucella suis,* and *Brucella melitensis,* causative agents of bovine brucellosis, are unwanted, notifiable organisms in New Zealand.

(2) In the interest of alignment with the recommendations of the OIE *Code*, the second option in the IRA was modified to require maintenance of semen collection centre freedom from brucellosis in accordance with the recommendations of the OIE *Code* chapter on collection and processing of bovine semen.

(3) Bovine brucellosis is not classified as a hazard in bovine embryos.

5.11.3 Recommendation

(1) The semen donor must be kept since birth in a country or zone that is free from *Brucella* in accordance with the OIE *Code*; or

(2) The semen collection centre must be maintained free from *Brucella* from commencement until conclusion of semen collection for export to New Zealand, through compliance with the recommendations in the OIE *Code* in relation to *Brucella*.

a) The centre must require that:

i) Prior to pre-entry isolation the donors must be either from a country or zone that is free from *Brucella* in accordance with the OIE *Code* or must be from a herd officially free from *Brucella*;
ii) During the 30 days prior to pre-entry isolation, donors must be tested with a test listed in *MPI-STD-TVTL* for *Brucella*, with negative results;
iii) All cattle in pre-entry isolation must be tested with a test listed in *MPI-STD-TVTL* for *Brucella*, with negative results;
iv) At least annually all cattle resident in the semen collection centre must be tested with a test listed in *MPI-STD-TVTL* for *Brucella*, with negative results.

5.12 *Campylobacter fetus* subspecies *venerealis* (bovine genital campylobacteriosis, BGC)

5.12.1 Risk management options presented in the 2018 risk work

(1) Veterinary Authorities of importing countries should require the presentation of an international veterinary certificate attesting that the donor animals:

a) Have never been used for natural service; or
b) Have only mated virgin heifers; or
c) Were kept in an artificial insemination centre where no case of bovine genital campylobacteriosis (BGC) has been reported; and
d) The culture of semen and preputial specimens for the presence of the causal agent of bovine genital campylobacteriosis proved negative.

Note: This option aligns with the recommendations provided by OIE *Code*. Some of the included sub options may be difficult to certify. The option should only be considered where BGC is notifiable.

(2) The semen was collected, processed and stored in accordance with Chapters 4.6 and 4.7 of the OIE *Code*; and

a) The donor animal has been kept in a pre-entry isolation facility for at least 28 days and after a minimum of 7 days in pre-entry isolation has undergone testing for *Campylobacter fetus* subspecies *venerealis* as follows:
b) Animals less than six months old or kept since that age only in a single sex group prior to pre-entry isolation should be tested once on a preputial specimen, with a negative result.
c) Animals aged six months or older that could have had contact with females prior to pre-entry isolation should be tested three times at weekly intervals on a preputial specimen, with a negative result in each case.
d) All resident bulls and teasers in the semen collection facility are tested at least annually for *Campylobacter fetus* subspecies *venerealis*, with negative results, where the country or zone where the semen collection facilities are located is not free;

i) A preputial specimen should be tested.
ii) Only bulls on semen production or having contact with bulls on semen production need to be tested. Bulls returning to collection after a lay-off of more than six months should be tested not more than 30 days prior to resuming production.

Note: This aligns with the recommendations for the importation of bovine semen provided by OIE *Code* Chapter 4.6.

5.12.2 Discussion

(1) *Campylobacter fetus* subspecies *venerealis*, which is the causative organism of bovine genital campylobacteriosis (BCG), was endemic in New Zealand and the 2009 IRA did not recommend risk mitigation.

(2) There have been no laboratory confirmed isolations of *Campylobacter fetus* subspecies *venerealis* in New Zealand since 1992 (Loveridge & Gardner 1993)[3].

(3) *Campylobacter fetus* subspecies *venerealis* is notifiable in New Zealand under the Biosecurity (Notifiable Organisms) Order 2016.

(4) The IRA 2018 estimated that the likelihood of entry of *Campylobacter fetus* subspecies *venerealis* in *in vivo* derived bovine embryos, processed as per the IETS protocols is negligible thus the risk estimate for embryos is assessed to be negligible. Accordingly the IRA 2018 concluded that risk management measures for *Campylobacter fetus* subspecies *venerealis* in imported bovine embryos are not required. The OIE *Code* also does not recommend any measures for bovine embryos.

(5) BGC is an OIE listed disease (OIE 2018) and the OIE *Code* chapter 4.6 recommends testing bulls and teaser animals used for producing semen for *Campylobacter fetus* subspecies *venerealis*. Chapter 11.3, Article 4, provides specific recommendations for bovine semen imports.

(6) The IRA 2018 assessed *Campylobacter fetus* subspecies *venerealis* in semen to be a risk and risk management is scientifically justified.

(7) In recommending risk management options for *Campylobacter fetus* subspecies *venerealis* in bovine semen, the IRA 2018 considered that:

a) *Campylobacter fetus* subspecies *venerealis* is transmitted through natural service and AI using infected bull semen.
b) Infection in bulls is not associated with clinical signs, gross lesions, histological changes or altered semen quality.
c) In 2011 susceptibility to antibiotics generally used in semen treatment was limited to gentamicin.

(8) The *Code's* requirement that donors "were kept in an establishment or artificial insemination centre where no case of bovine genital campylobacteriosis has been reported" is challenging to confirm and is not expected to improve risk management.

5.12.3 Recommendation

(1) The semen requirements should align with the recommendations of the OIE *Code*. Which includes Chapters 4.6 and 4.7 and Article 11.3.4.

(2) The semen donor must never have been used for natural service; or

a) must have only been mated virgin heifers.

(3) After a minimum of 7 days in pre-entry isolation, the semen donor must undergo testing for *Campylobacter fetus* subspecies *venerealis* as follows:

a) Animals less than six months old or kept since that age only in a single sex group prior to pre-entry isolation must be tested with test listed in *MPI-STD-TVTL*, once on a preputial specimen, with a negative result.
b) Animals aged six months or older that could have had contact with females prior to pre-entry isolation must be tested with an approved test three times at weekly intervals on preputial specimens, with a negative result in each case.
c) Annual testing:

[3] Loveridge, R., Gardner, E., (1993) Campylobacter fetus venerealis infection in cattle, Surveillance Surveillance Vol.20 No.4.

i) A preputial specimen/s from donor bulls and any in-contact animals on semen production must be tested with an approved test or culture.
ii) Bulls returning to collection after a lay-off of more than six months must be tested not more than 30 days prior to resuming production.

5.13 *Chlamydophila abortus*

5.13.1 Risk management options presented in the 2009 IRA

(1) Donors resident since birth or for the previous 2 years in a country or zone free from *Chlamydophila abortus* based on no laboratory confirmation of infection in any species for at least 2 years.

(2) Individual donors could be tested serologically using an OIE recommended test for *C. abortus*, 2-3 weeks after germplasm collection.

(3) Aliquots of germplasm could be tested for *C. abortus* by PCR or antigen detection ELISA, with negative results.

(4) Tetracycline or macrolide antibiotics could be added to imported germplasm.

5.13.2 Discussion

(1) The literature on *C. abortus* infection in cattle does not provide evidence of transmission from cattle to other susceptible animal species or humans. The literature indicates that *C. abortus* has been associated with abortion in cattle, but transmission appears to be limited to periods of close association between cattle and infected sheep or goats, or where bedding from animals infected with *C. abortus* is recycled in dairy herd housing.

(2) In one study it was shown that infected ewes did not produce embryos that led to infection of recipients or progeny. There have not been studies to assess transmission from infected bovine embryos, but it is considered unlikely and there are no documented cases of transmission.

(3) Experimental studies have shown that *C. abortus* can be excreted in semen of inoculated bulls and isolation of the agent from semen of naturally infected bulls and rams has been reported. Transmission from semen has not been demonstrated.

(4) While sheep are considered long term carriers of *C. abortus*, it is unknown if cattle can be long term carriers of infection and if they would harbour the organism in the reproductive tract.

(5) All human cases of abortion caused by *C. abortus* have been associated with direct contact with small ruminants.

(6) Establishment in New Zealand would have significant impacts on sheep farming and human health.

(7) It was concluded in 2010 that measures specifically against *Chlamydophila* should not be placed in the import health standard for bovine germplasm.

5.13.3 Recommendation

(1) Measures are not justified.

5.14 *Coxiella burnetii* (Q-fever)

5.14.1 Risk management options presented in the 2009 IRA

(1) Require acaricide treatment and subsequent inspections to demonstrate tick freedom during the 30 day quarantine of germplasm donors.

(2) Donors could be tested by an ELISA test, with negative results, 21-60 days after the final collection of germplasm for export to New Zealand. Consider prohibiting germplasm from any animals that have ever been known to test positive.

(3) MPI approved test on each collection of germplasm.

5.14.2 Discussion

(1) Acaricide treatment and 30 day quarantine in tick free premises would add considerable cost to the import of bovine germplasm.

(2) Serological testing of donors has been used historically to mitigate risk. The IDEXX ELISA test has been validated for cattle and shown to provide acceptable sensitivity; other ELISA tests will require MPI approval during veterinary certificate negotiation.

(3) There was a 2016 study indicating that the indirect fluorescence antibody (IFA) assay may be a superior option for testing ruminants.

(4) The IHS should be written to allow for MPI approval of other test methods in the future.

(5) An option for whole herd testing for Q fever was incorporated in the 2011 IHS. This option is useful for countries conducting herd monitoring on a routine basis.

5.14.3 Recommendation

(1) The germplasm donor must never have been confirmed positive for Q fever; and either

a) The donor must be subjected to a serological test listed in *MPI-STD-TVTL* for Q fever, on a sample collected between 21 and 120 days after each germplasm collection for export to New Zealand, with negative results; or

b) An aliquot of semen or a sample of embryos/oocytes, collection fluids and/or washing fluids from each germplasm collection for export to New Zealand must be tested for Q fever with a test listed in *MPI-STD-TVTL*, with negative results; or

c) Within the 6 month period before or after germplasm collection for New Zealand, but before export, the embryo collection herd or semen collection centre herd must be tested for Q fever, with negative results. This testing must be with a test listed in *MPI-STD-TVTL* and must be performed on either the whole herd or a random sample of at least 60 animals (whichever is the lesser number); and

i) The herd must be isolated for the period between semen collection and diagnostic sampling.

5.15 *Mycobacterium* tuberculosis (bovine tuberculosis)

5.15.1 Risk management options presented in the 2009 IRA ~~for bovine germplasm~~

(1) Semen donors could be required to show no clinical sign of bovine tuberculosis on the day of collection of the semen.

(2) Semen donors could be required to be kept in an artificial insemination centre free from bovine tuberculosis in a country, zone or compartment free from bovine tuberculosis and which only accepts animals from free herds in a free country, zone or compartment as defined by the OIE.

(3) Semen donors could be required to show negative results to tuberculin tests carried out annually and be kept in a herd free from bovine tuberculosis as defined by the OIE.

(4) Embryo donors and all other susceptible animals in the herd of origin could be required to show no clinical sign of bovine tuberculosis during the 24 hours prior to embryo collection.

(5) Embryo donors could be required to have originated from a herd free from bovine tuberculosis in a country, zone or compartment free from bovine tuberculosis.

(6) Embryo donors could be required to be kept in a herd free from bovine tuberculosis, be isolated in the establishment of origin for the 30 days prior to departure to the collection centre, and be subjected to a tuberculin test for bovine tuberculosis with negative results.

5.15.2 Discussion

(1) In accordance with suggested options in the import risk analysis, alignment with the OIE *Code* and the New Zealand bovine tuberculosis pest management strategy was chosen for management of the risk associated with bovine tuberculosis for bovine germplasm imported to New Zealand.

5.15.3 Recommendation

(1) Semen:

a) The semen collection centre must be:

i) free from bovine tuberculosis in accordance with the OIE *Code*;
ii) located in a country or zone that has been recognised by the CTO as free from bovine tuberculosis; or

b) The semen collection centre must be maintained free from bovine tuberculosis from commencement until conclusion of semen collection for export to New Zealand, through compliance with the recommendations in the OIE *Code* in relation to bovine tuberculosis; and

i) Prior to pre-entry isolation, donors must be from a herd free from bovine tuberculosis, either in accordance with the OIE *Code* or the competent authority of the exporting country;
ii) During the 30 days prior to entry to the semen collection centre, donors must be tested with a test listed in *MPI-STD-TVTL* for bovine tuberculosis, with negative results;
iii) At least annually all resident cattle were tested with a test listed in *MPI-STD-TVTL* for bovine tuberculosis, with negative results.

(2) Embryos:

a) No clinical signs of bovine tuberculosis were observed in the embryo collection herd during the 24 hours prior to embryo collection for export to New Zealand; and either

i) The donors must be:

1) from an embryo collection herd that is free from bovine tuberculosis in accordance with the OIE *Code* or the competent authority of the exporting country;
2) from a country or zone that has been recognised by the CTO as free from bovine tuberculosis; or

ii) The donor must be:

1) from an embryo collection herd that is free from bovine tuberculosis, either in accordance with the OIE *Code* or the competent authority of the exporting country;
2) subjected to a test listed in *MPI-STD-TVTL* for bovine tuberculosis during the period between 30 days prior to and 12 months after embryo collection for export to New Zealand, with negative results.

5.16 *Mycoplasma mycoides* subsp. *mycoides* SC (contagious bovine pleuropneumonia, CBPP)

5.16.1 Risk management options presented in the 2009 IRA

(1) Donors of germplasm could be required to originate from a country or zone that is free from CBPP.

(2) Donors could be required to not be vaccinated against CBPP and be kept since birth or for at least 6 months in an establishment where no case of CBPP has been reported and the establishment is not situated in a CBPP infected zone.

(3) Donors could be subjected to an OIE recommended serological test with negative results on two occasions 21-30 days apart with the last test done within 14 days prior to germplasm collection.

5.16.2 Discussion

(1) CBPP is caused by *Mycoplasma mycoides* subsp. *mycoides* SC

(2) For the purposes of the OIE *Code*, the incubation period is 6 months. Studies have shown the incubation period to be between 3 weeks and 3 months.

(3) Each of the options presented in the import risk analysis were included, in modified form, in the recommended options for the 2011 IHS. The second and third options were included as a combination of requirements for countries infected with CBPP.

5.16.3 Recommendation

(1) The germplasm donor must be born in and have been continuously resident in a country that is recognised by the CTO as free from CBPP; or

(2) The germplasm donor must:

a) never have been vaccinated for CBPP;
b) be kept since birth, or for at least the 6 months prior to commencement until conclusion of germplasm collection for export to New Zealand in establishments where no case of CBPP has been reported, and which are not situated in a CBPP infected zone, as defined by the OIE *Code*;
c) be serologically tested for CBPP, using a test listed in *MPI-STD-TVTL* on two occasions 21 to 30 days apart, with the last test within 14 days prior to germplasm collection for export to New Zealand, with negative results.

5.17 Other mollicutes (*Mycoplasma* and *Ureaplasma* spp.)

5.17.1 Risk management options presented in the 2009 IRA

(1) The 2009 IRA recommended the following options for management of Mollicutes (*Mycoplasma bovigenitalium, Mycoplasma bovis, Mycoplasma verecundum, Mycoplasma californicum, Mycoplasma canadense, Mycoplasma group 7* and *Ureplasma diversum*).

a) Addition of an appropriate combination of antibiotics to semen extenders.
b) Culture of germplasm for identification of organisms before adding antibiotics.
c) Culture of germplasm for identification of organisms after addition of antibiotics.

5.17.2 Discussion

Ureaplasmas

(1) Ureaplasmas are present in New Zealand, however it is unknown which species are present.

(2) Measures are not considered justified.

Mycoplasma bovis risk management since 2011

(3) The OIE *Code* does not have a chapter on *Mycoplasma* spp. and there is no reporting requirement.

(4) The current *M. bovis* requirement was incorporated in 2011. It indicates that the donor has never recorded a positive test for *M. bovis.* It does not require *M. bovis* testing. MPI has considered the *M. bovis* risk managed via the requirement that donors (and the semen collection centres or collection herd) had to meet the general health requirements as set out by the OIE *Code* and semen and embryos had to be collected and processed in accordance with the OIE *Code*, including specifications for the addition of antibiotics.

(5) The OIE *Code's* recommended antibiotic combinations are (antibiotic dose is per mL frozen semen):

a) Gentamicin (250 µg), tylosin (50 µg), lincomycin–spectinomycin (150/300 µg) (GTLS);
b) Penicillin (500 IU), streptomycin (500 µg), lincomycin-spectinomycin (150/300 µg);
c) Amikacin (75 µg), divekacin (25 µg).

(6) The OIE *Code* does not recommend a specific protocol for the use of antibiotics during semen or embryo processing.

(7) The OIE *Code* references the International Embryo Technology Society (IETS) Manual for embryo processing and antimicrobial use. An effective approach to managing *M. bovis* contamination is discussed in the IETS *Manual*, but it is not specifically recommended by IETS and would not have been carried out on exported embryos under the current terms of trade since targeted antibiotic treatment is not explicitly stated in the IHS.

Discussion Document: Mycoplasma bovis Risk Management in Bovine Germplasm

(8) The document: *Discussion Document: Mycoplasma bovis Risk Management in Bovine Germplasm* ended on 5 November 2019. It:

a) explains the eradication programme and why MPI attributes New Zealand's *M. bovis* freedom until 2017, after decades of imports of over 2.5 million straws, to be due to the low risk of transmission and use of antibiotics;
b) communicates that it is unknown how *M. bovis* entered New Zealand;
c) discusses semen processing and antibiotic treatment, as well as other risk mitigation strategies such as testing.

Antibiotic treatment of semen and embryos

(9) The USA's Certified Semen Services (CSS) claims that their antibiotic protocol ('Minimum Requirements") is effective against mycoplasmas and ureaplasmas. This is based upon a 1988 study conducted on inoculated semen. The antibiotic options recommended by the OIE (other than the one that matches CSS) are not supported by literature as effective for *M. bovis* control.

(10) *CSS Minimum Requirements Appendix 1* provides two methods for their antibiotic procedures. The Alternative CSS Protocol (1-Step Method) is preferred because the GTLS antibiotics in each ml of extender is the same as that prescribed for neat semen treatment in the 2-Step Method (i.e., 100 µg tylosin, 500 µg gentamicin, 300/600 µg Linco-Spectin) and yet the final concentration of antibiotics is doubled compared to 2-Step Method.

(11) MPI considers there to be a lack of research comparing the two methods and how effective they are when various types of extenders are used. Doubling the antibiotic concentrations and requiring a set of minimum requirements for the treatment protocol ensures that this risk management approach is as effective as possible, while allowing the industry to make their own decision about which type of extender is used.

(12) As discussed in *Discussion Document: Mycoplasma bovis Risk Management in Bovine Germplasm*, several studies have shown that *M. bovis* is developing greater antimicrobial resistance. Doubling the dose of antibiotics in semen and following the protocol for embryos as described in the IETS *Manual* is therefore an appropriate strategy for managing the risk in future imports.

(13) At this time there are no studies conclusively demonstrating *M. bovis* transmission from naturally infected, processed germplasm. The minimum number of organisms required to result in infection via this pathway is therefore also unknown.

(14) Considering the significant uncertainty regarding *M. bovis* transmission potential via germplasm, effective management of the risk should be considered achieved with the use of a targeted antibiotic protocol which has been demonstrated to significantly reduce the viability of the organisms in the sample.

Testing for *M. bovis*

(15) Experimentally, *M. bovis* antibodies can be detected in serum by indirect ELISA 6 to 10 days after infection. However under natural conditions, individual animal titres are not well correlated with infection or disease, meaning antibodies may not be detected in infected animals. Moreover, the ELISA test is validated as a herd detection tool rather than for use in individual animals and its estimated diagnostic sensitivity is around 75%.

(16) Low diagnostic sensitivity of the available tests and the persistent asymptomatic infection with intermittent shedding of *M. bovis* do not permit screening of donors by serological tests to confirm freedom from infection.

(17) The major challenge to current molecular detection tests is the intermittent shedding of *M. bovis*. PCR results cannot be considered a reliable indicator of freedom from infection with *M. bovis*.

(18) The *Discussion Document: Mycoplasma bovis Risk Management in Bovine Germplasm* mentions a validated PCR test for semen, however validation is incomplete and the sensitivity of the PCR test is unknown.

New Zealand's *M. bovis* status

(19) Genetic analysis of recovered *M. bovis* strains provide evidence that *M. bovis* was not endemic prior to the initial detection in 2017 and all isolates sequenced to date are consistent with a single strain being introduced into New Zealand in 2015/16.

(20) Surveillance data shows that most new infected properties are identified through tracing, rather than general surveillance, signalling that *M. bovis* has not become endemic and that multiple geographically widespread entry events or systematic failure had not occurred. Surveillance data supports the findings of the genetic analysis.

5.17.3 Recommendation

Either (a) or (b) below is required for semen and embryos as indicated below:

(1) Collection and processing of germplasm must be in accordance with the recommendations of the OIE *Code*, except, for:

(2) Semen:

a) The following antibiotic combination must be used at the specified final dose per mL of extended semen:

i) Gentamicin (500 µg), tylosin (100 µg), lincomycin–spectinomycin (300/600 µg) (GTLS); or

1) Another MPI approved antibiotic combination, listed in *MPI-STD-TVTL*;

ii) Antibiotics must be prepared and stored as separate stock solutions as described by the manufacturer to maintain potency;

iii) Antibiotics must be added to media/extender on the day of processing;

iv) The semen must remain in the antibiotic solution at the recommended concentration for a minimum of 2 hours at no less than 5°C before being frozen in the antibiotic solution; or

b) Another MPI approved antibiotic combination and protocol, listed in *MPI-STD-TVTL*.

(3) Embryos:

a) The embryos must be subjected to the protocol described in the IETS *Manual*: tylosin (200 µg/mL) incubation at 37°C in the antibiotic treatment for a minimum of 4 hours after being washed 10 times; or

b) The embryos must be subjected to another MPI approved antibiotic combination and protocol, listed in *MPI-STD-TVTL*; or

(4) Each germplasm collection for export to New Zealand must be tested with a validated PCR test for *M. bovis* listed in *MPI-STD-TVTL*, with negative results.

5.18 Exotic *Leptospira* spp. (leptospirosis)

5.18.1 Risk management options presented in the 2009 IRA

(1) Serological testing of donors to demonstrate freedom from exotic *Leptospira* spp.

(2) PCR or culture of germplasm for exotic *Leptospira* spp.

(3) Preparation of germplasm in accordance with the recommendations of the OIE *Code*, including the use of suitable antibiotics in semen diluents and embryo washing media.

5.18.2 Discussion

(1) *Leptospira* spp. are sensitive to a variety of antibiotics and treatment of the animal or inclusion of antibiotics in prepared semen has traditionally been used to prevent dissemination of *Leptospira* spp. by international trade. Antibiotic treatment of embryos is likely to be effective. No further requirements are needed other than certification that germplasm has been collected and processed as per the *Code* and/or the IETS *Manual*. Specific requirements for *Leptospira* spp. are not needed as it does not provide any further risk reduction.

(2) The *Code* chapter for leptospirosis was removed as it did not meet the criteria for listing. Requirements for leptospirosis were removed from the live horse IHS in a previous amendment. Scientific justification is lacking for requirements because the maintenance hosts for some exotic serovars are unknown. The import of live animals is associated with a greater risk than the import of germplasm, and therefore requirements should also be removed for bovine germplasm.

5.18.3 Recommendation

(1) It is proposed that no measures be placed in the standard specifically against exotic *Leptospira* spp.

5.19 Exotic *Salmonella* spp. (salmonellosis)

5.19.1 Risk management options presented in the 2009 IRA ~~for bovine germplasm~~

(1) Require official certification that donors originate from farms on which outbreaks of salmonellosis have not occurred in the previous 3 years.

(2) Require culture of semen or embryos or wash fluid sediment. Where pathogenic *Salmonella* spp. exotic to New Zealand is isolated, importation of germplasm could be prohibited.

5.19.2 Discussion

(3) Property freedom would be difficult to certify.

(4) Culture of germplasm after addition of antibiotics is the only option that would be practically feasible since culture prior to addition of antibiotics would require preparation of germplasm specifically for New Zealand. This would however significantly reduce access to genetics while increasing costs.

(5) Culture of germplasm after addition of antibiotics would have questionable sensitivity because organisms would be present at low levels. In addition, culture of germplasm for *Salmonella* spp. would add significantly to importation costs.

(6) Historically risk from contamination of imported bovine germplasm with Salmonella spp. has been accepted, and *Salmonella* spp generally require higher levels of contamination than would be likely to be present in germplasm, and an oral route of entry, to be infective.

(7) For these reasons, it was concluded that measures specifically against salmonellosis should not be placed in the import health standard for bovine germplasm.

5.19.3 Recommendation

It is proposed that no measures be placed in the standard specifically against exotic *Salmonella* spp.