Ministry for Primary Industries
Manatū Ahu Matua

Risk Management Proposal

Semen and Embryos from Horses (*Equidae*)

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New Zealand Government

Growing and Protecting New Zealand

Disclaimer

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1 Purpose

The purpose of this document is to:

- Show how options for the management of risk organisms in semen and embryos from horses have been assessed
- Provide recommendations for import requirements for semen and embryos from horses.

The import health standard (IHS) for semen and embryos from horses from specified countries is developed under Section 24 of the Biosecurity Act 1993.

For a detailed risk assessment of the identified hazards, refer to the *Import Risk Analysis: Equine germplasm from Australia, Canada, the European Union and the USA* (22 December 2009). A copy can be viewed at the following link: http://www.biosecurity.govt.nz/files/regs/imports/risk/equine-germplasm-ra.pdf

2 Background

Equine semen and embryos are considered risk commodities, with the potential to harbour exotic viral, bacterial and protozoal diseases. In December 2009, the Ministry for Agriculture and Forestry (now the Ministry for Primary Industries, MPI) completed the *Import Risk Analysis: Equine germplasm from Australia, Canada, the European Union and the USA*. This import risk analysis (2009 IRA) forms the basis of the risk management measures in the proposed IHS.

Norway requested inclusion into the new IHS for semen and embryos from horses. A comparison of the Norwegian animal health situation (WAHID 2014) with that of EU member countries has supported the decision to allow imports of equine semen and embryos from Norway. Similarly, Switzerland was included after comparing the animal health situation with that of other EU countries. See Table 1 for more information.

The following IHSs for individual countries or groups of countries will be consolidated into one IHS which will contain generic import requirements for semen and embryos:

- *Import Health Standard for Horse Semen from Australia, 10 November 2004*
- *Import Health Standard for Horse Semen from Canada, 8 August 2005*
- *Import Health Standard for Equine Semen from the European Union, 1 August 2007*
- *Import Health Standard for Horse Semen from the USA, 12 February 2009.*

In addition to the IHS, a guidance document for equine semen and embryos will be issued. This document will include model veterinary certificates.

Table 1: Organisms of concern in semen and embryos of horses.

Hazard	Transmissible in semen/ embryos	Present in Australia?	Present in EU?	Present in Norway?	Present in Switzerland?	Present in North America?	Transmitted only by insect or helminth vectors♥	Preliminary hazard for 2009 IRA
Viruses								
African horse sickness virus	Yes%/Yes%	No*	No*	No*	No*	No*	Yes@	No
Vesicular stomatitis virus	Yes/Yes"	No*	No*	No*	No*	Yes*	Yes@	No
Venezuelan equine encephalomyelitis virus	Yes"/Yes"	No*	No*	No*	No*	Not since 1971*	Yes@	No
Eastern equine encephalomyelitis virus	No/No	No*	No*	No*	No*	Yes*	Yes@	No
Western equine encephalomyelitis virus	No/No	No*	No*	No*	Not in 2013*	Yes*	Yes@	No
Equine infectious anaemia virus	Yes/Yes	Yes*	Yes*	Not since 1975*	Not in 2013, last report unknown*	Yes*	Mechanical insect vectors also in semen	Yes
Equine influenza virus	No/No	No[1]	Yes*	Yes (2008)*	Yes (2009)*	Yes*	No@	Yes
Equine herpesvirus-1 (exotic strains)	Yes/No	Yes*	Yes*	Yes (2006)*	Yes*	Yes*	No@	Yes
Equine arteritis virus	Yes/No	Yes*	Yes*	Yes*	Yes*	Yes*	No@	Yes
Horse pox viruses	No/No	No*	No*	No*	No	No*	No@	No
Japanese encephalitis virus	No/No	No*[2]	No*	No*	No*	No*	Yes@	No
West Nile virus	No/No	No**	Yes**	No*	No*	Yes*	Yes@	No
Rabies virus	No/No (uncertainty)	No*	Yes*	Yes?*	Last rep in 2003*	Yes*	No@	Yes
Borna disease virus	No/Yes	No**	Yes**	Yes**	Yes	No**	No@	Yes
Equine encephalosis virus	No/unknown	No**	No**	No*	No	No@	Yes@	No
Louping ill virus	No/unknown	No**	Yes**	Yes	Yes	No[3]	Yes@	No
Hendra virus	Yes/Yes	Yes**	No**	No	No	No**	No@	Yes
Getah virus	No/unknown	No**	No**	No	No	No**	Yes[@]	No
Bacteria								
Bacillus anthracis	Yes/Yes	Yes*	Yes*	Yes (1993)*	Yes (1997)*	Yes*	No@	Yes
Leptospira spp.	Yes/Yes	Yes*	Yes*	Yes	Yes*	Yes*	No@	Yes
Taylorella equigenitalis	Yes/No	No*	Yes*	Yes	Not in 2013* (last rep in 2008)	Yes*	No@	Yes
Taylorella asinigenitalis								
Burkholderia mallei	No/No	No*	No*	Last reported 1889	Last reported 1939	No*	No@	No
Burkholderia pseudomallei	No/Yes	Yes*	No*	No*	No	No[4]	No@	Yes
Salmonella spp.	Yes/Yes	Yes*	Yes*	Yes	Not in 2013	Yes*	No@	Yes
Other organisms								
Ehrlichia risticii and *Ehrlichia equi*	No/No	No**	Yes**	Not in 2013	Not in 2013	Yes	Yes@	No
Histoplasma capsulatum var. *farciminosum*	No/No	No*	No*	No*	No	No*	No*	Yes#

Other organisms from IRA 2000								
Bovine brucellosis *(B. abortus)*	Yes/No	No*	Some countries*	No	Last in 1996*	No (Can), Yes (USA)*	No	Yes
Lyme disease	No/No	Yes	Yes	Yes?	Yes?	Yes	Yes	No
Q fever	Yes/No	Yes*	Yes*	No	Yes	Yes*	Yes@	No
Equine protozoal myeloencephalitis (*Sarcocystis neurona*)	No/No	Yes?	No	No	No	Yes	Yes	No
Dourine	Yes/No	No*	Italy*	No	Not in 2013*	No*	No	Yes
Equine piroplasmosis	No/No	Not in 2013*	Some countries*	No	Yes*	Yes*	Yes	No
Nipah	Yes/unknown	No*	No*	No	No*	No*	Yes	No
Surra	No/No	No*	Not in 2013*	No	No*	No*	Yes	No

* Country status as reported on the World Animal Health Information Database (OIE 2013).
** Disease distribution discussed in the MAF *Import risk analysis: horses and horse semen* (MAF 2000a) and cross-referenced in the textbook *Veterinary Medicine* (10th Ed) (Radostits et al 2007).
% found in semen but no transmission evidence - To date there have been no known outbreaks due to the use of infected semen, ova or embryos.
@ As reported by the appropriate authors and in the appropriate sections In: Infectious Diseases of Livestock (Authors-various 2004).
# Although not known to occur in the relevant countries it is hard to be certain as the organism may be found in soil mud etc.
♥ The organisms marked "yes" in column 8 are transmitted only by arthropod vectors or in one case a helminth vector (*Ehrlichia risticii*). These organisms are not transmissible via germplasm and therefore they are not considered to be hazards in the commodity.

3 Objective

The objective is to manage, to an appropriate level of protection, the biosecurity risks for equine semen and embryos. Measures will align with the OIE *Code* when possible.

The biosecurity risks associated with the importation of equine semen and embryos have been identified in the 2009 IRA. Of the potential hazards identified in equine semen and embryos, risk management measures are justified for the following:

Viral
Equine arteritis virus, OIE listed disease; unwanted, notifiable organism
Equine herpesvirus-1 (exotic strains), OIE listed disease
Equine infectious anaemia virus, OIE listed; unwanted, notifiable organism

Bacterial
Leptospira spp (exotic serovars), other exotic organisms
Taylorella equigentialis, unwanted, notifiable organism.

Borna disease and equine salmonellosis were identified as potential hazards, but no measures for these diseases were warranted when importing semen and embryos of horses.

4 General Requirements

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

The *Code* provides methods to prevent germplasm from being contaminated with risk organisms during collection, processing, storage, and transport. Although the *Code's* methods for embryos are not specific to horses, they have been adapted for horses in this document. The measures describe procedures for entry of a semen donor into a semen collection centre, which involves testing the donor prior to pre-entry isolation, testing during the isolation period, and annual testing on the centre. In the case of embryo collection, the *Code* describes three phases of risk: the health status of the herd or country of origin, the processes followed during collection of embryos, and the testing of embryos collected or surveillance of the donors.

It is recommended that the embryo collection team should be approved by the Competent Authority. The team's practices, as well as the processing and storage of embryos and any test samples, should be in accordance with the *Code* where applicable.

If testing before collection is permitted, the donor should be required to be isolated from other animals not of equivalent tested health status until the end of germplasm collection. On the day of collection, the health status of each donor should be confirmed to be free of clinical evidence of infectious diseases transmissible in germplasm by the approved veterinarian. Additionally, if risk is managed by ensuring absence of disease from the donor and establishment for a specific amount of time after collection, the germplasm should be stored for the amount of time specified under each disease before export. All germplasm should be stored in sealed, tamper-proof, labelled straws or containers.

The IETS *Manual* provides guidance regarding washing and verification of the intact zona pellucida (intact and free of adherent material) after washing. These are important steps in processing to ensure that viruses will not be transmitted with the embryo. Micro-manipulation should only be permitted after washing and verification of the zona pellucida for maximum assurance that the embryo is free of virus.

5 Recommendations for Identified Risk Organisms

5.1 Borna disease virus

5.1.1 Options for management of risk presented in the 2009 IRA for equine germplasm

Option 1

i. Both male and female donors could come from countries certified by the veterinary authority as free from the disease; or
ii. In countries where the disease does occur, and in which the disease is notifiable, animals could be certified as having been resident for the previous 3 months on a property on which the disease has not occurred during the previous 12 months.

NB. this option is similar to the requirements in the current IHS for the importation of horses.

Option 2

Aliquots of semen or a sample of embryos and embryo washing solution from each batch of germplasm could be tested by RT-PCR for Borna disease virus RNA, with negative results.

NB. This test is not validated for this purpose but could be justified on the grounds of probable high sensitivity. However, it is unlikely to be available in most testing laboratories.

Option 3

Aliquots of semen or a sample of embryos and embryo washing fluid from each batch of germplasm could be cultured on cell cultures derived from embryonic rabbit or rat brain with negative results

NB. This method is unlikely to be available in many (possibly most) testing laboratories.

Option 4

Aliquots of semen and a sample of each batch of embryos could be tested by intracerebral inoculation of rabbits, with negative results.

NB. This test may be unacceptable on animal ethics grounds and unlikely to be available in many laboratories.

5.1.2 Discussion

Borna disease virus is listed on the Unwanted Organisms Register as an exotic organism. It is not an OIE listed disease.

The requirements for Borna disease in the current IHS for horses (HORANIIC.GEN, 2014) are as follows, similar to Option 1:

i. horses were kept since birth or for at least the past 90 days in a free country; or
ii. horses were kept since birth or for at least the past 90 days on premises in which no case has been reported during the past 12 months.

There are no measures for Borna virus in the current IHSs for equine semen. Borna disease is not OIE-listed and is not important to trade. It occurs only sporadically in horses and sheep, and exceptionally in a variety of animals in parts of Germany. The epidemiology is not fully understood, but current understanding is that there is reasonable evidence that this disease is spread by direct contact or fomites. There is no evidence that it is transmitted venereally or by traded germplasm. This goes for all species. Accordingly, any measures for Borna disease are difficult to scientifically justify.

5.1.3 Recommendation

It is recommended no measures are put in place for Borna disease.

5.2 Equine herpesvirus-1 (EHV-1)

5.2.1 Options for management of risk presented in the 2009 IRA for equine germplasm:

For Semen

Option 1

Semen could be imported provided that donor stallions complied with the *Code* requirement for live horses.

Option 2

Semen could be imported provided each batch of semen was tested by a RT-PCR test for the neuropathogenic mutant strain of EHV-1 with negative results.

For Embryos

Under the assumption that trypsin washing will be effective in removing any EHV-1 from equine embryos, embryos could be imported provided trypsin washing is used in addition to the existing International Embryo Transfer Society requirements for equine embryos.

5.2.2 Discussion

Although EHV-1 and EHV-4 are present in New Zealand, measures are recommended to prevent the introduction of exotic, pathogenic strains of EHV-1. EHV has been reported in both equine semen and embryos and no country approved for export of semen and/or embryos to New Zealand is free of EHV-1.

Trypsin may have an adverse effect on embryo viability. The current IHS for horses (2013) requires that the horses meet the measures recommended in the OIE *Code*. As imported horses pose a greater risk of introducing EHV than semen and embryos, the risk associated with imported semen and embryos should be effectively managed when derived from donors that have complied with the OIE *Code* requirements for live horses.

Current OIE Code recommendations for the importation of equines

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

1. *showed no clinical sign of equine herpes virus type 1 infection (abortigenic and paralytic forms) on the day of shipment and during the 21 days prior to shipment;*

2. *were kept for the 21 days prior to shipment in an* establishment *where no* case *of equine herpes virus type 1* infection *(abortigenic and paralytic forms), was reported during that period.*

5.2.3 Recommendation

Donor animals must meet the OIE *Code* recommendations for managing EHV-1 in horses.

5.3 Equine infectious anaemia virus (EIA)

5.3.1 Options for management of risk presented in the 2009 IRA for equine germplasm:

For Semen

Option 1

Semen could be imported provided that:

i. donors show no clinical sign of EIA on the day of semen collection and for the 60 days after semen collection: and
ii. no case of EIA has been associated with any premises where the animals were kept during the 3 months prior to and the 60 days after semen collection.

Option 2

Semen could be imported provided that:

i. donors show no clinical sign of EIA on the day of semen collection; and
ii. no case of EIA has been associated with any premises where the donors were kept during the 3 months prior to collection; and
iii. Donors are subjected to an AGID test or ELISA for EIAV antibody not less than 21 days after entry onto the semen collection centre with a negative result.

(Risk analysis subsequently recommended that the timing of the test be modified to not less than 30 days after entering the semen collection centre, to allow time for antibody development.)

Option 3

Semen could be imported provided that:

i. *donors show no clinical sign of EIA on the day of semen collection and for the 60 days after semen collection; and*
ii. *no case of EIA has been associated with any premises where the animals were kept during the 3 months prior to and the 60 days after semen collection; and*
iii. *donors are subjected to an AGID test or ELISA for EIAV antibody 30-60 days after semen collection with negative results.*

NB. In most cases antibody can be detected by the AGID within 45 days of infection however, although rare, the period between exposure and antibody detection can be as long as 60 days.

For Embryos

Option 1

Embryos could be imported provided that:

i. *both male and female donors show no clinical sign of EIA on the day of semen collection and for the 60 days after germplasm collection: and*
ii. *no case of EIA has been associated with any premises where the donor animals were kept during the 3 months prior to and the 60 days after semen collection.*

Option 2

Embryos could be imported provided that:

i. *both male and female donors show no clinical sign of EIA on the day of germplasm collections and for the 60 days after germplasm collection; and*
ii. *no case of EIA has been associated with any premises where the animals were kept during the 3 months prior to and the 60 days after germplasm collection; and*
iii. *donors are subjected to an AGID test or ELISA for EIAV antibody 30-60 days after germplasm collection with negative results.*

5.3.2 Discussion

EIA is an OIE listed disease and the virus is an unwanted, notifiable organism in New Zealand. The virus has been shown to be transmitted by semen. There is uncertainty regarding the risk of transmission via embryos. There are OIE *Code* requirements for live horses, but none for semen and embryos.

The 2014 IHS for horses requires that horses meet the OIE *Code*, with samples for testing collected in pre-export isolation or in the 21 days prior to export if PEI is not required. Option 2 for semen and option 2 for embryos are similar to the OIE *Code* for horses.

Current OIE Code recommendations for the importation of horses

Veterinary Authorities of importing countries should require the presentation of an international veterinary certificate attesting that:

1. *the* animals *showed no clinical sign of equine infectious anaemia (EIA) on the day of shipment and during the 48 hours prior to shipment; and*

2. *no* case *of EIA has been associated with any premises where the* animals *were kept during the three months prior to shipment; and*

3. *if imported on a permanent basis, the* animals *were subjected to a diagnostic test for EIA with negative results on blood samples collected during the 30 days prior to shipment; or*

4. *if imported on a temporary basis, the* animals *were subjected to a diagnostic test for EIA with negative results on blood samples collected during the 90 days prior to shipment.*

5.3.3 Recommendation

Donors must meet the OIE *Code* recommendations for managing EIA in horses.

5.4 Equine arteritis virus (Equine viral arteritis, EVA)

5.4.1 Options for management of risk presented in the 2009 IRA for equine germplasm

For Semen

Donor stallions should conform to the recommendations of Article 12.10.4 of the *Code*. However, the *Code* recommendations are complex and contain a number of different options.

For Embryos

Option 1

Embryos could be imported provided that:

i. *male donors meet the requirements in the relevant Articles of the Code; and*
ii. *female donors have been vaccinated with an approved vaccine according to the manufacturer's recommendations, at least 4 weeks before collection of embryos.*

Option 2

Embryos could be imported provided that:

i. *male donors meet the requirements in the relevant Articles of the Code; and*
ii. *female donors are subjected to a serological test 1 week before and 3 weeks after collection of embryos. Female donors would be suitable donors if both serological tests are negative or if positive titres are stable or declining.*

5.4.2 Discussion

EVA is an OIE listed disease and the virus is listed as an unwanted, notifiable organism in New Zealand. In 2014 New Zealand was recognised by the OIE as free from EVA. The virus is known to be shed in semen and stallions can remain carriers of the virus. The OIE *Code* recommendations for semen will cover this risk.

Transmission through embryos is unknown, and mares do not remain carriers of the virus. Options 1 & 2 for female donors are similar to the OIE *Code* recommendations for the importation of equids other than uncastrated males, with the timing modified for embryo collection. However, since the 2009 IRA was written, the OIE *Code* has recommended measures for trade in equine embryos.

Current OIE Code recommendations for the importation of equine semen

Veterinary Authorities of importing countries should require the presentation of an international veterinary certificate attesting that the donors were kept for the 28 days prior to semen collection in an establishment where no equid has shown any clinical sign of EVA during that period and showed no clinical sign of EVA on the day of semen collection; and

1. *were subjected between six and nine months of age to a test for EVA as prescribed in the OIE Manual:*

   Either;

   a) *with a negative result, or*

b) *with a positive result, followed at least 14 days later by a second test showing a stable or decreasing titre;*

and were immediately vaccinated against EVA and regularly revaccinated according to the recommendations of the manufacturer; or

2. *were isolated and not earlier than seven days of commencing isolation were subjected to a test for EVA as prescribed in the OIE Manual on a blood sample with negative results, immediately vaccinated for EVA, kept for 21 days following vaccination separated from other equids and regularly revaccinated according to the recommendations of the manufacturer; or*
3. *were subjected to a test for EVA as prescribed in the OIE Manual on a blood sample with negative results within 14 days prior to semen collection, and had been separated from other equids not of an equivalent EVA status for 14 days prior to blood sampling until the end of semen collection; or*
4. *have been subjected to a test for EVA as prescribed in the OIE Manual carried out on a blood sample with positive results and then: either*

    a) *were subsequently test mated to two mares within six months prior to semen collection, which were subjected to two tests for EVA as prescribed in the OIE Manual with negative results on blood samples collected at the time of test mating and again 28 days after the test mating; or*
    b) *were subjected to a test for equine arteritis virus as prescribed in the OIE Manual with negative results, carried out on semen collected within six months prior to collection of the semen to be exported; or*
    c) *were subjected to a test for equine arteritis virus as prescribed in the OIE Manual with negative results, carried out on semen collected within six months after the blood sample was collected, then immediately vaccinated, and revaccinated regularly; or*

5. *for frozen semen, were subjected with negative results either:*

    a) *to a test for EVA as prescribed in the OIE Manual carried out on a blood sample taken not earlier than 14 days and not later than 12 months after the collection of the semen for export; or*
    b) *to a test for equine arteritis virus as prescribed in the OIE Manual carried out on an aliquot of the semen collected immediately prior to processing or on an aliquot of semen collected within 14 to 30 days after the first collection of the semen to be exported.*

Current Code recommendations for the importation of in vivo derived equine embryos

Veterinary Authorities of importing countries should require the presentation of an international veterinary certificate attesting that the donor animals showed no clinical sign of EVA on the day of embryo collection; and

EITHER

1. *were kept in an establishment where no animals have shown any signs of EVA for the 28 days prior to collection; and*
    a. *were subjected to a test for EVA carried out on blood samples collected either once within 21 days prior to collection with negative results, or on two occasions at least 14 days apart within 28 days prior to collection, which demonstrated stable or declining antibody titres; or*
    b. *were regularly vaccinated according to the recommendations of the manufacturer;*

OR

2. *were isolated for the 28 days prior to collection and during this period the animals showed no sign of EVA;*

AND

3. *semen used to fertilise the oocytes complies with the requirements in the Code Article relevant to EVA in semen.*

5.4.3 Recommendation

Semen

Donors must meet the OIE *Code* recommendations for managing EVA in equine semen.

Embryos

Donors must meet the OIE *Code* recommendations for managing EVA in *in vivo* derived embryos.

5.5 *Leptospira* species

5.5.1 Options for management of risk presented in the 2009 IRA for equine germplasm:

Option 1

Donor horses could be tested serologically with a variety of antigens that occur in the exporting country and not in New Zealand, with negative results.

Option 2

Donor horses could be treated with effective antibiotics within one week prior to germplasm collection.

Option 3

Diluents containing antibiotics that are effective against *Leptospira* spp. could be used in the preparation of the semen and antibiotics could be included in the solutions used in the preparation of embryos.

NB: this reflects the recommendations of the IETS Manual and the current IHSs for horse semen from countries covered by this risk analysis.

5.5.2 Discussion

The OIE *Code* does not make any recommendations for leptospirosis. *Leptospira* species are sensitive to a variety of antibiotics, and treatment of animals or inclusion of antibiotics in prepared semen has traditionally been used to prevent dissemination of *Leptospira* species by international trade. Treatment of embryos is also likely to be effective.

The June 2009 *Review of leptospirosis measures in Import Health Standards* (p.13, recommendation 2) recommends that semen and embryos be prepared according to the OIE *Code* and International Embryo Transfer Society (IETS) standard.

> **The OIE *Manual*** has a chapter on "Collection and processing of bovine, small ruminant and porcine semen", but none specifically for equine semen, which includes the following:
>
> *A mixture of antibiotics should be included with a bactericidal activity at least equivalent to that of the following mixtures in each ml of frozen semen: gentamicin (250 µg), tylosin (50 µg), lincomycin–spectinomycin (150/300 µg); penicillin (500 IU), streptomycin (500 µg), lincomycin-spectinomycin (150/300 µg); or amikacin (75 µg), divekacin (25 µg).*

From *IETS Manual* Ch 7:

Penicillin (100 IU/mL) and streptomycin (100 µg/mL) or gentamycin (50 µg/mL) have been used most commonly to prevent bacterial replication during culture of bovine embryos.

Leptospira borgpetersenii serovar hardjobovis has been found associated with washed oocytes and embryos, supplementation of the media with penicillin and streptomycin will ensure that embryos are not contaminated with infectious bacteria.

Current IHS for equine semen

An effective combination of antibiotics was added to the semen extender/diluent. The combination must produce an effect at least equivalent to the following:

500 IU per ml streptomycin; or
500 IU per ml penicillin; or
150 µg per ml lincomycin; or
300 µg per ml spectinomycin; or
50 µg per ml gentamycin.

Additionally, equivalence has been given for Ticarcillin at 1.2 mg/ml and Amikacin 0.5 mg/ml (equals 1.0 mg per ml of Timentin). The antibiotics are listed in the MPI-Document for *Approved Diagnostic Tests, Vaccines, Treatments and Post-Arrival Testing Laboratories for Animal Import Health Standards* (MPI-STD-TVTL).

5.5.3 Recommendation

Semen and embryos must be prepared with antibiotics effective against *Leptospira* species as listed in MPI-STD-TVTL.

5.6 *Salmonella* species

5.6.1 Options for management of risk presented in the 2009 IRA for equine germplasm:

Option 1

i. *The donors were kept for the 3 months prior to collection on premises where salmonellosis has not occurred during that period; and*
ii. *The horses were showing no clinical signs of salmonellosis on the day of collection.*

NB: this option reflects the current import health standards for equine semen.

Option 2

i. *donors could be resident for at least 3 months on properties on which no cases of salmonellosis have been diagnosed during the previous 3 years; and*
ii. *faecal samples from donors could be cultured according to methods recommended in the OIE Manual of Diagnostic Tests and Vaccines (Davies 2008), three times at weekly intervals immediately before germplasm collection, with negative results.*

NB. The 3 year property freedom period and the intervals and frequency of sampling are conservative, albeit arbitrary.

Option 3

Aliquots of each semen and embryo batch to be imported could be cultured, using methods described in the OIE Manual of Diagnostic Tests and Vaccines, with negative results.

5.6.2 Discussion

Salmonella spp. (exotic affecting animals) are listed as exotic, unwanted organisms. There are no OIE *Code* measures recommended for *Salmonella* in horses or semen or embryos. While *S. abortus equi* is thought to be transmitted venereally, the likelihood of introducing exotic salmonellae in equine collected, processed and stored following OIE *Code* recommendations is considered remote. There is no evidence that Salmonellosis has been transmitted via semen or embryos collected and processed in such a manner.

Option 1 is consistent with the current requirements for importing horses:

> *The horses were showing no clinical signs of equine Salmonellosis at the final inspection prior to departure and were kept for at least the past 90 days on premises where no case of equine Salmonellosis (S. abortus equi) has been reported during that time.*

Option 2 is consistent with the current OIE *Code* in applying no measures.

5.6.3 Recommendation

It is recommended no measures regarding equine salmonellosis are applied to either semen or embryos.

5.7 *Taylorella* species (Contagious equine metritis, CEM)

5.7.1 Options for management of risk presented in the 2009 IRA for equine germplasm:

Option 1

Donors of germplasm could be required to be resident for at least 2 months on a property that is not considered to be an infected establishment according to the *Code* definition.

Option 2

a) Donors of germplasm could be required to be resident for at least 2 months on a property that is not considered to be an infected establishment according to the Code definition; and
b) Swabs from the donors could be tested, with negative results, by culturing or PCR on three occasions. Once within 7 days prior to germplasm collection and twice at weekly intervals during the 21 days after germplasm collection. The swabs could be collected from the prepuce, urethral sinus and fossa glandis (including the diverticulum) of stallions and from the mucosal surfaces of the urethra, clitoral sinuses and clitoral fossa in the case of mares.

Option 3

Germplasm could be sourced from any donors provided semen diluents and solutions used for preparation of embryos contain antibiotics that are effective against *Taylorella* spp.

Option 4

Germplasm could be imported from countries in which CEM occurs, but is notifiable, provided that donors have been resident for at least 3 months on a property on which no case of CEM has been found for at least 3 years.

Option 5

Germplasm imports could be restricted to donors from countries that are considered free from CEM on the basis that the disease is notifiable and has not occurred in the previous 3 years.

5.7.2 Discussion

Taylorella equigenitalis is an unwanted, notifiable organism. The OIE *Manual* identifies this as the causative agent of contagious equine metritis (CEM). As the organism is known to be transmitted venereally, measures are justified. The *Manual* also notes that *T. asinigenitalis* has been identified in male donkeys, mares and stallions, and "would appear to be passed to other donkeys and horses during mating". It has not been associated with naturally occurring disease. A PCR test is required to distinguish between these two species.

A recent review of CEM measures for the revision of the Import Health Standard for Horses (1 February 2013) resulted in a recommendation to align with the current OIE *Code* and *Manual*.

The *Manual* recommends culture as the method of identification, which does not distinguish between the two species of *Taylorella*. MPI has approved quantitative polymerase chain reaction (qPCR) as a testing method in horses. This recommendation will be adopted in the semen and embryos standards as well.

The 2012 *OIE Code* has the following measures for live horses:

Recommendations for the importation of stallions and mares considered free from CEM (for countries where an official control organisation is present)

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

1. *showed no clinical sign of CEM on the day of shipment;*
2. *have had no contact with CEM:*
    a. *directly, through coitus with an infected animal; or*
    b. *indirectly, by passing through an infected establishment;*
3. *were subjected to the laboratory test for CEM with negative results during the 30 days prior to shipment.*

Article 12.2.3.

Recommendations for the importation of stallions and mares which have previously shown signs of CEM or which have been in contact with CEM (for countries where an official control organisation is present)

Veterinary Authorities of importing countries should require the presentation of an international veterinary certificate attesting that the animals which have been in direct contact through coitus with an infected animal, or indirect contact by passing through an infected establishment:

1. *have been recognised as not being contagious through laboratory tests for CEM;*
2. *have been protected against any possibility of contagion since the beginning of the tests.*

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Either

a. *were from a CEM-free country, approved by MPI; or*
b. *were considered free from CEM; an official control programme for CEM or MPI-approved equivalent is established in the country of export; and horses have met the recommendations as described in the OIE Code and OIE Manual (or pre-mating testing followed recommendations in the OIE Manual but was done in the 60 days prior to mating or artificial insemination); or*
c. *have been known to be infected with CEM and were subject to an effective method of treatment and testing approved by MPI.*

5.7.3 Recommendation

Donors must meet the OIE *Code* recommendations for managing CEM in horses.