



Risk Management Proposal

Semen and Embryos from Equids

EQUIGERM.SPE

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1 Purpose

The purpose of this document is to:

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

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 (IRA) 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 import health standard (IHS).

Norway requested inclusion into the new IHS for semen and embryos from horses. A comparison of the Norwegian animal health situation 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 have been consolidated into one IHS which will contain generic import requirements for semen and embryos from those approved countries:

- *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.*

Where equids are required to meet OIE Terrestrial Code (*Code*) recommendations in the IHS, the requirements reflect the current *Code* as of 2018. When *Code* chapters are amended, MPI will review these changes to ensure they continue to align with New Zealand's appropriate level of protection. Where *Code* recommendations no longer meet New Zealand's appropriate level of protection, they will be replaced with risk-based MPI recommendations and the IHS will be amended. Otherwise the most recent version of the *Code* should be referred to.

The IHS contains import requirements to manage the biosecurity risk of importing equine semen and embryos from Australia, Canada, the European Union, Norway, Switzerland, and the United States of America. The IHS serves as the basis for country to country (bilateral) negotiations of country specific veterinary certificates. A guidance document will be issued by MPI and this will provide commodity specific guidance information including samples of country specific bilaterally-agreed veterinary certification for trade in equine semen and embryos.

3 Objective

The objective is to effectively manage biosecurity risks associated with the import of equine semen and embryos consistent with New Zealand's domestic legislation and international obligations.

4 Options assessment

Under Article 3.3 of the World Organisation for Animal Health (OIE) Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement), risk management measures which provide a level of protection greater than provided by international standards may be imposed only when they can be scientifically justified on the basis of a risk assessment.

For a detailed analysis of potential hazards and their risks please refer to the supporting documents, *Import Risk Analysis: Equine Germplasm from Australia, Canada, the European Union and the USA* which contains the relevant risk assessment and an analysis of management options for each risk organism.

Of the potential hazards, the *IRA Equine Germplasm from Australia, Canada, the European Union and the USA* concluded that risk management measures were justified for the following hazards in imported equine semen and embryos:

- Borna disease virus
- Equine arteritis virus (equine viral arteritis)
- Equine herpesvirus-1 (abortigenic and paralytic forms)
- Equine infectious anaemia virus
- *Leptospira* spp (exotic serovars)
- *Salmonella abortus equi*
- *Taylorella equigenitalis* and *Taylorella asinigenitalis* (contagious equine metritis)

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 and they were not included in the previous IHS.

Identified risk organisms that have been added in the 2018 major amendment to the *IHS: Semen and Embryos from Equids*:

- *Trypanosoma equiperdum* (dourine)

Identified risk organisms that have been removed in the 2018 major amendment to the IHS Semen and Embryos from Equids:

- *Leptospira* spp

5 General requirements for all importations of equine semen and embryos

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 Technology Society (IETS).

5.1 Application

- (1) The scope of the commodity includes semen and embryos from horses (*Equus caballus*) and donkeys (*Equus asinus*). These are listed in the IHS in *Part 1*.
- (2) The IHS applies to equine semen and embryos for import from approved countries into New Zealand which includes Australia, Canada, the European Union, Norway, Switzerland, and the USA. These countries are listed in the IHS in *Part 1*.

5.2 Diagnostic tests, vaccines, and treatments

- (1) All testing required by the IHS must be conducted at a laboratory approved to conduct export testing by the Competent Authority of an approved country.
- (2) MPI approved diagnostic tests must be either described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (the *Manual*) (<http://www.oie.int/en/standard-setting/terrestrial->

- [manual/access-online/](#)) or will only be approved after consultation with MPI laboratory experts. When tests are not as per the *Manual* they must be considered by the MPI Animal Health Laboratory (AHL) as valid for diagnostic purposes in equids and must be appropriate for surveillance for the identified risk organism.
- (3) All diagnostic tests and vaccines must be approved by the CTO and listed in the document, [Approved Diagnostic Tests, Vaccines, Treatments and Post-arrival Testing Laboratories for Animal Import Health Standards \(MPI-STD-TVTL\)](#).
 - (4) All products and vaccinations administered to meet the specific disease requirements in *Part 2* of the IHS must be administered according to the recommendations of the manufacturer in a country that the CTO has agreed meets the requirements for export to New Zealand. All vaccinations must be either the final dose of a primary vaccination course or the recommended booster to complement the primary course.
 - (5) Organism testing for frozen semen can be valid from the date of entry to the semen collection centre until a maximum of 180 days from the date the sample was collected for a single test, or the date of the final test in that series (e.g. CEM) provided:
 - a) The donor remains continuously resident at the semen collection centre;
 - b) The semen collection centre remains under the supervision of the Competent Authority;
 - c) All equids at the semen collection centre remain free from evidence of infectious disease transmissible in equine germplasm;
 - d) While resident at the semen collection centre, donors have only had contact with equids of equivalent tested health status;
 - e) The donors have not been mated by natural service after entering the semen collection centre and during the collection period;
 - f) If any of the above provisions cease to apply or if more than 180 days have passed since the sample collection date, the donors and all in contact equids at the semen collection centre must be re-tested in accordance the requirements of the IHS.

5.3 General donor requirements

- (1) Donors must be isolated from other equids not of an equivalent tested health status from the commencement of testing until the completion of germplasm collection for export to New Zealand.
- (2) On the day of collection an approved veterinarian must perform an examination of the donor and any teaser animals, including the external reproductive organs, to ensure the donor and teaser animal is free from clinical evidence of infectious disease transmissible in germplasm.
- (3) Teaser animals used for the collection of semen must be of equivalent tested health status to donor animals.

5.4 Semen requirements

- (1) Semen must be non-genetically modified.
- (2) Semen collection and processing centres must meet the recommendations of the relevant articles of the *Code* chapter *General Hygiene in Semen Collection and Processing Centres*.
- (3) The semen must be collected in a semen collection centre that is approved by the Competent Authority prior to the start of each period of collection of semen for export, or where the centre is used continuously, on an annual basis. Approval must be no more than 12 months before the last date of collection of semen in the consignment.
- (4) Semen collection, processing, and storage must be conducted at a standard equivalent to the general considerations for hygienic collection and handling of semen that is specified in the *Code* chapter *Collection and Processing of Bovine, Small Ruminant and Porcine Semen*.
- (5) The 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.

- (6) Semen must be in straws, or new or disinfected containers which are sealed and tamper-evident, and clearly and permanently marked to identify the donor and the date(s) of collection. If a code is used for this information, its decipher instructions must accompany the consignment.

5.5 Embryo requirements

- (1) Embryo donors must not be situated on premises or with other equids that are subject to veterinary restrictions for the identified risk organisms managed in the IHS for at least 28 days prior to the first embryo collection until completion of donor testing.
- (2) Embryos must be *in vivo* derived, frozen, non-cloned, and non-genetically modified.
- (3) The embryo collection team must the recommendations of the *Code* chapter *Collection and Processing of In-Vivo Derived Embryos from Livestock and Equids*.
- (4) Embryo collection, processing, and storage must the recommendations of the *Code* chapter *Collection and Processing of In-Vivo Derived Embryos from Livestock and Equids*.
- (5) Embryos must have an intact zona pellucida and be free of adherent material after the final wash when examined over its entire surface at not less than 50X magnification. If any micro-manipulation is done that causes a breach of the zona pellucida, it must be done according to the procedures described in the *Code* and *IETS Manual*.
- (6) The 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.
- (7) Embryos must be in new or disinfected containers which are sealed and tamper-evident, and clearly and permanently marked to identify the donor and the date(s) of collection. The marking must conform to the international standards of the IETS. If a code is used for this information, its decipher instructions must accompany the consignment.
- (8) The semen used to artificially inseminate the donor mare must meet all semen requirements of this IHS.

5.6 Storage

- (1) Semen and embryos may only be stored with semen or embryos of equivalent health status and that have been collected and processed in compliance with the *Code* and the IETS. Containers must be held in a storage place approved by the Competent Authority of the exporting country until the time of export.
- (2) Storage of semen and embryos in a third country (other than the country of origin) is permitted if the third country is a specified country listed in this IHS.

5.7 Transport

- (1) All transport containers in which semen or embryos are transported to New Zealand must be new or disinfected and must be free of contamination.
- (2) The transport container in which semen and embryos are transported to New Zealand must be sealed, by either the approved veterinarian or an Official Veterinarian, using tamper-evident seals.

6 Recommendations for identified risk organisms

6.1 Borna disease virus

6.1.1 Risk management options presented in the IRA 2009

Option 1

- (1) *Both male and female donors could come from countries certified by the veterinary authority as free from the disease; or*
- (2) *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

- (1) *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

- (1) *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

- (1) *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.

6.1.2 Discussion

Borna disease (BD) is an endemic, sporadically occurring disease caused by Borna disease virus (BDV). Borna disease is not an OIE listed disease. Clinically manifest BD is endemic in Central Europe (Germany, Switzerland, Austria, Liechtenstein), but infection has also been recognised in France and Sweden.

There are no measures for Borna virus in the current or previous IHSs for equine germplasm. It is assumed that intranasal infection via the olfactory nerve is the natural route of infection. There is reasonable evidence that this disease is spread by direct contact with infected horses or fomites. There is no evidence that BDV is transmitted venereally or has ever been spread by internationally traded equine germplasm.

6.1.3 Recommendation

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

6.2 Equine arteritis virus

6.2.1 Risk management options presented in the IRA 2009

For Semen

- (1) *Donor stallions should conform to the recommendations for the importation of equine semen in the Code chapter for Infection with equine arteritis virus.*

For Embryos

Option 1

- (1) *Embryos could be imported provided that:*
 - a) *male donors meet the requirements in the relevant Articles of the Code; and*
 - b) *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

- (1) *Embryos could be imported provided that:*
 - a) *male donors meet the requirements in the relevant Articles of the Code; and*
 - b) *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.*

6.2.2 Discussion

Equine viral arteritis (EVA) is an OIE listed disease and equine arteritis 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 effectively manage this risk. An additional testing option has been included from the OIE *Code* recommendations when importing horses as this will also manage the risk when importing equine germplasm.

Transmission through embryos is unknown, and mares do not remain carriers of the virus. However, since the 2009 IRA was written, the OIE *Code* has recommended measures for trade in equine embryos which are acceptable.

6.2.3 Recommendation

- (1) Semen donors:
 - a) Must meet the recommendations for managing equine semen in the *Code* chapter *Infection with Equine Arteritis Virus*; or
 - b) Must be kept for the 28 days prior to semen collection on premises where no equid has shown any clinical sign of EVA during that period show no clinical sign of EVA on the day of semen collection; and
 - i) Must be subjected to a test for EVA carried out on a single blood sample collected not less than 21 days after entry into the semen collection centre.
- (2) Embryo donors must meet the recommendations for managing *in vivo*-derived embryos in the *Code* chapter *Infection with Equine Arteritis Virus*.

6.3 Equine herpesvirus-1

6.3.1 Risk management options presented in the IRA 2009

For Semen

Option 1

- (1) *Semen could be imported provided that donor stallions:*
 - a) *Were kept for the 21 days prior to semen collection in an establishment where no case of equine herpesvirus type 1 infection was reported during that period; and*

- b) *Showed no clinical signs of EHV-1 infection on the day of collection and during at least the 21 days the semen is stored prior to shipment*

NB This option is essentially the same as the recommendations for horses in the OIE Code, which does not provide any safeguard against importing semen from donors that are latent carriers.

Option 2

- (1) *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. Identification of EHV-1 could be followed by more detailed examination to distinguish between EHV-1B and EHV-1P. Semen infected with EHV-1B could be disqualified.*

For Embryos

- (1) *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.*

6.3.2 Discussion

EHV-1 is an OIE listed disease. The *Code* provides recommendations for EHV-1 (abortigenic and paralytic forms) for live equids only.

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.

The current IHS for horses (2015) requires that the horses meet the measures recommended in the OIE *Code*. As imported horses pose a greater risk of introducing EHV-1 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 equids. Some studies have reported negative effects on equine embryos that have undergone trypsin washing, and there is little knowledge about its ability to mitigate risk for EHV-1. Furthermore, EHV-1 is endemic in New Zealand and hence the import measures imposed on germplasm should not be more stringent than those imposed on imported live horses to prevent the introduction of exotic pathogenic strains.

6.3.3 Recommendation

- (1) Semen and embryos donors:
- a) Must be kept on premises where no case of EHV-1 (abortigenic and paralytic forms) has been reported in the 21 days prior to each collection; and
 - b) Must show no clinical signs of EHV-1 infection on the day of collection and during the 21 days prior to collection.

6.4 Equine infectious anaemia virus

6.4.1 Risk management options presented in the IRA 2009

For Semen

Option 1

- (1) *Semen could be imported provided that:*
- a) *donors show no clinical sign of EIA on the day of semen collection and for the 60 days after semen collection; and*

- b) *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

- (1) *Semen could be imported provided that:*
 - a) *donors show no clinical sign of EIA on the day of semen collection; and*
 - b) *no case of EIA has been associated with any premises where the donors were kept during the 3 months prior to collection; and*
 - c) *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

- (1) *Semen could be imported provided that:*
 - a) *donors show no clinical sign of EIA on the day of semen collection and for the 60 days after semen collection; and*
 - b) *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*
 - c) *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

- (1) *Embryos could be imported provided that:*
 - a) *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*
 - b) *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

- (1) *Embryos could be imported provided that:*
 - a) *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*
 - b) *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*
 - c) *donors are subjected to an AGID test or ELISA for EIAV antibody 30-60 days after germplasm collection with negative results.*

6.4.2 Discussion

EIA is an OIE listed disease and the equine infectious anaemia virus (EIAV) 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 to ensure the safe international movement of horses, but there are no recommendations made when trading equine semen and embryos.

Studies on other lentiviruses in regards to embryos show that the interaction of the virus with oocytes and the standard embryo processing procedures for *in vitro* embryos (which are a higher risk commodity than *in vivo* embryos) prevents lentivirus transmission (<https://doi.org/10.1016/j.theriogenology.2011.08.019>).

The 2015 IHS for horses requires that samples for testing are collected in pre-export isolation or in the 21 days prior to export if PEI is not required. The application of premises freedom and testing no less than 21 days after entry into the collection centre or within the 21 days prior to collection of embryos along with OIE-recommended collection and processing protocols would be sufficient to manage the risk of introducing EIAv transmission in both semen and embryos.

6.4.3 Recommendation

- (1) Semen and embryo donors:
 - a) Must be kept on premises where no case of EIA has been reported during the 90 days prior to each collection; and
 - b) Must show no clinical sign of EIA on the day of each collection; and
 - c) Must be tested for EIA not less than 21 days after entry into the collection centre for semen or in the 21 days prior to collection for embryos, with a negative result.

6.5 *Leptospira* species

6.5.1 Risk management options presented in the IRA 2009

Option 1

- (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

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

Option 3

- (1) 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.

6.5.2 Discussion

The OIE *Code* does not make any recommendations for leptospirosis. At the OIE General Session in May 2009, the International Committee accepted the recommendation that the empty *Code* chapter on leptospirosis should be deleted.

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. Treatment of embryos is also 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, and antibiotics have been added to the germplasm in generally accepted levels. Specific requirements for *Leptospira* spp. are not needed as it does not provide any further risk reduction.

Requirements for leptospirosis were removed from the live horse IHS in a previous amendment. The import of live animals is associated with a greater risk than the import of germplasm, and therefore specific leptospirosis requirements should also be removed for equine semen and embryos.

6.5.3 Recommendation

It is recommended that no specific measures are put in place for leptospirosis.

6.6 *Salmonella abortus equi*

6.6.1 Risk management options presented in the IRA 2009

Option 1

- (1) *The donors were kept for the 3 months prior to collection on premises where salmonellosis has not occurred during that period; and*
- (2) *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

- (1) *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*
- (2) *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

- (1) *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.*

6.6.2 Discussion

Equine salmonellosis is not an OIE-listed and not many countries impose import measures mitigating the organism. This organism is rarely encountered in developed countries. Infected stallions exhibit clinical signs related to the male reproductive organs such as epididymitis and orchitis, and it would be very unlikely that a stallion with these clinical signs would be certified as clinically healthy and accordingly eligible to be utilised as a donor. There would also be potential issues with the viability and quality of the semen.

While *S. abortus equi* is thought to be transmitted venereally, the likelihood of introducing exotic salmonellae in equine germplasm collected from clinically healthy donors and then processed following OIE *Code* recommendations, including the addition of antibiotics during processing, is considered remote. There is no evidence that equine salmonellosis has been transmitted via semen or embryos collected and processed in such a manner. Other than certification that germplasm has been collected and processed as per the *Code* and/or the IETS manual, and antibiotics have been added to the germplasm in generally accepted levels, no further requirements are needed.

6.6.3 Recommendation

It is recommended that no specific measures are put in place for *Salmonella abortus equi*.

6.7 *Taylorella equigenitalis* and *Taylorella asinigenitalis* (contagious equine metritis)

6.7.1 Risk management options presented in the IRA 2009

Option 1

- (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

- (1) *Donors of germplasm could be required to be resident for at least 2 months on a property that not considered to be an infected establishment according to the Code definition; and*
- (2) *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 surface of the urethra, clitoral sinuses and clitoral fossa in the case of mares.*

Option 3

- (1) *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

- (1) *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

- (1) *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.*

6.7.2 Discussion

Contagious equine metritis (CEM) is an OIE listed disease and the *Code* makes recommendations for the safe importation of stallions and mares but not germplasm. As the organism is known to be transmitted venereally, measures are justified. To align with the OIE *Code* where possible, the measures imposed on semen and embryos from horses should reflect the recommendations for importing horses as described in the *Code*. It also recommends an establishment can be declared free two months after the confirmation of the last case and after thorough cleaning and disinfection of the premises. The *Manual* briefly describes characteristics of a control programme, based on import and export testing and testing for breeding populations.

6.7.3 Recommendation

It is recommended that semen and embryo donors meet the measures reflecting the *Code* recommendations for managing CEM in live equids:

- (1) Semen and embryo donors must be kept, since birth or for at least the 60 days prior to collection, in a country recognised by the CTO as free from CEM, where no case of CEM has been reported in the 2 years prior to export; or
- (2) Semen and embryo donors must be kept, since birth or for at least 60 days prior to collection, on premises where no case of CEM has been reported during that time; and
 - a) Must have no direct or indirect contact with CEM during the 60 days prior to collection; and
 - b) Must not show no clinical sign of CEM on the day of each collection; and

- c) Must be subjected to a test for CEM listed in MPI-STD-TVTL not less than 7 days after entry into the collection centre for semen, or in the 30 days prior to collection for embryos, with negative results;
 - i) Stallions must be sampled two times at intervals of 4-7 days. Swab sampling sites are the urethra; urethral fossa and its sinus; and the penile sheath.
 - ii) Mares must be sampled two times at intervals of 4-7 days. Swab sampling sites are the clitoral fossa and sinuses; and
 - d) Must not receive antibiotics in the 7 days (systemic treatment) or 21 days (local treatment) before the first sample collection or during the CEM sampling period; or
- (3) Donors that have previously shown signs of CEM or have been in direct or indirect contact with CEM during the two months prior to collection:
- a) Must be treated for CEM; and
 - b) After treatment, must be subjected to a test for CEM listed in MPI-STD-TVTL, with negative results:
 - i) Stallions must be sampled three times at intervals of at least 7 days (sampling sites are the urethra, urethral fossa and its sinus, and the penile sheath). Thereafter, the first three mares mated or inseminated by the stallion are tested on clitoral swabs taken 3 times at intervals of at least 7 days, starting 2 days after mating or insemination.
 - ii) Mares must be sampled three times at intervals of at least 7 days (sampling sites are the clitoral fossa and sinuses), and 3 endometrial swabs taken during the next 3 oestrus periods. Maiden mares only require 1 endometrial swab; and
- (4) Must be protected against any possibility of infection with CEM since the beginning of the tests.

6.8 *Trypanosoma equiperdum* (dourine)

6.8.1 Risk management options presented in the IRA 2000

Trypanosoma equiperdum was not considered in the IRA 2009. The risk management options for horse semen in the IRA 2000 for horses and horse semen are as follows:

Option 1:

- (1) *The donor stallions were kept since birth, or for the 6 months prior to collection, in a country that has been free from dourine for the past 6 months according to the criteria within Article 3.4.2.2. of the OIE Code.*

Option 2:

- (1) *The donor stallions were kept for the 6 months prior to collection on premises where dourine has not occurred during that period; and*
- (2) *The donor stallions were subjected to the CFT or c-ELISA for dourine with negative results: Either:*
- a) *prior to collection, and from the period 30 days prior to testing until collection has not naturally mated any mares not of the equivalent health status; Or:*
 - b) *not less than 30 days following collection of semen for export; and*
- (3) *The donor stallions were showing no clinical sign of dourine on the day of collection; and*
- (4) *Semen for export has been examined microscopically prior to freezing and no trypanosomes were detected.*

6.8.2 Discussion

Dourine is an OIE listed disease caused by the protozoan parasite *Trypanosoma equiperdum*. Equids are the reservoir host for dourine. The incubation period, severity and duration of disease vary considerably; it is often fatal, but spontaneous recoveries may occur and latent carriers exist. Further, subclinical infections also occur. There is no treatment or vaccine for dourine.

Unlike other trypanosomal infections, it is sexually transmitted during natural mating or by artificial insemination (AI) with infected semen. Transmission from stallions to mares is more common, but mares can also transmit the disease to stallions. The organism can be found in the vaginal secretions of infected mares and the seminal fluid, mucous exudate of the penis, and sheath of stallions. Male donkeys can be asymptomatic carriers and sexually immature animals that become infected can transmit the organism when they mature. Rarely, infected mares pass the infection to their foals.

Dourine was once widespread, and has been eradicated from many countries but is still seen in horses in Asia, Africa, South America, southern and eastern Europe, Mexico and Russia, and was reported in June 2011 in Sicily and then just north of Naples, on the Italian mainland.

Because there is no vaccine or treatment for dourine, freedom from disease is the only way to prevent the disease. The *Code* recommends that semen should either be imported from countries free from dourine, or if imported from endemic countries, the donor animals should be free from clinical signs of disease and test negative for dourine.

6.8.3 Recommendation

- (1) Semen donors must meet the recommendations for managing equine semen in the *Code* chapter *Dourine*.
- (2) Embryo donors must:
 - a) Be kept since birth, or for the 180 days prior to collection of the embryos in a country which has been free from dourine for not less than the past 180 days; or
 - b) Be kept for the 180 days prior to collection of the embryos on premises where no case of dourine was reported during that period and subjected to a diagnostic test for dourine with negative results.