Import risk analysis: Equine germplasm from Australia, Canada, the European Union and the USA

DRAFT FOR PUBLIC CONSULTATION

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MAF Biosecurity New Zealand

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Approved for interdepartmental review

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Executive Summary

The risk analysis examines the biosecurity risks associated with the importation of frozen semen and *in vivo* derived frozen embryos of horses, donkeys and zebras (collectively referred to as equine germplasm) from Australia, Canada, the European Union and the United States of America.

The disease agents considered were those in the hazard list of the MAF risk analysis for horses and horse semen carried out in 2000. Organisms that are already present in New Zealand were excluded from further consideration. A number of organisms were excluded on the grounds that they are not transmitted in germplasm, including protozoa with complex life cycles, arthropod parasites, helminth parasites, and disease agents that are transmitted exclusively by arthropod vectors or helminth intermediates.

Thirteen exotic organisms or strains of organisms were assessed to be potential hazards and these were submitted to further detailed analysis. As a result, the risks were considered to be non-negligible for the following organisms:

Equine infectious anaemia virus
Equine herpesvirus-1
Equine arteritis virus
Borna disease virus
Exotic *Leptospira* serovars
Taylorella spp.
Exotic *Salmonella* serovars

These organisms were therefore classified as hazards in the commodities, and options for the efficient management of the risk have been suggested.

1. Introduction

This risk analysis has been developed in response to applications to import equine embryos from several countries. A previous MAF risk analysis that was carried out in 2000 considered live horses and semen, but not embryos. Therefore, while the primary need was an assessment of the risks posed by equine embryos, the opportunity was taken to reassess equine semen in light of information that has arisen since 2000. In addition, it was considered appropriate for reasons of efficiency to consider a number of other equine animals in addition to horses, as reflected in the commodity definition below.

2. Scope

This risk analysis is limited to the risks posed by infectious agents in the importation of equine embryos and semen (equine germplasm). As such, the analysis is limited to organisms as defined in the Biosecurity Act 1993, and genetic diseases and other risks that may be of commercial importance to importers are not assessed.

3. Commodity definition

The commodities are defined as *in vivo* derived frozen embryos and frozen semen, collected and processed in accordance with international guidelines set out in the World Organisation for Animal Health (OIE) *Terrestrial Animal Health Code,* from healthy equidae including horses (*Equus caballus*), donkeys (*Equus asinus*), and zebras (*Equus quagga*, *Equus grevyi* and *Equus zebra*) resident in Australia, Canada, the European Union (EU)* member states or the USA.

* The EU includes the following countries: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and United Kingdom

This risk analysis rests on the following internationally-accepted principles that underline the collection and storage of embryos and semen.

- Germplasm is collected and processed at suitable collection centres and laboratories approved for the purpose by the veterinary administration of the exporting country. The place where the collection occurs, the equipment used and the laboratory in which the germplasm is processed is of a standard equivalent to that specified for other animal species in chapters 4.5 and 4.7 of the OIE *Terrestrial Animal Health Code* (hereafter referred to as the *Code*) (OIE 2008a).

- Germplasm is collected only from clinically healthy donors.

- If any testing of germplasm is necessary to support certification of the health status of donors, it is carried out at a laboratory approved by the veterinary administration of the exporting country.

- Germplasm is processed, packaged, stored and transported according to standards laid down in the *Code* (OIE 2008a) and the Research Subcommittee of the International Embryo Transfer Society (IETS), Health and Safety Advisory Committee (Stringfellow and Seidel 1998), including the use of antibiotics and trypsin as recommended by IETS.

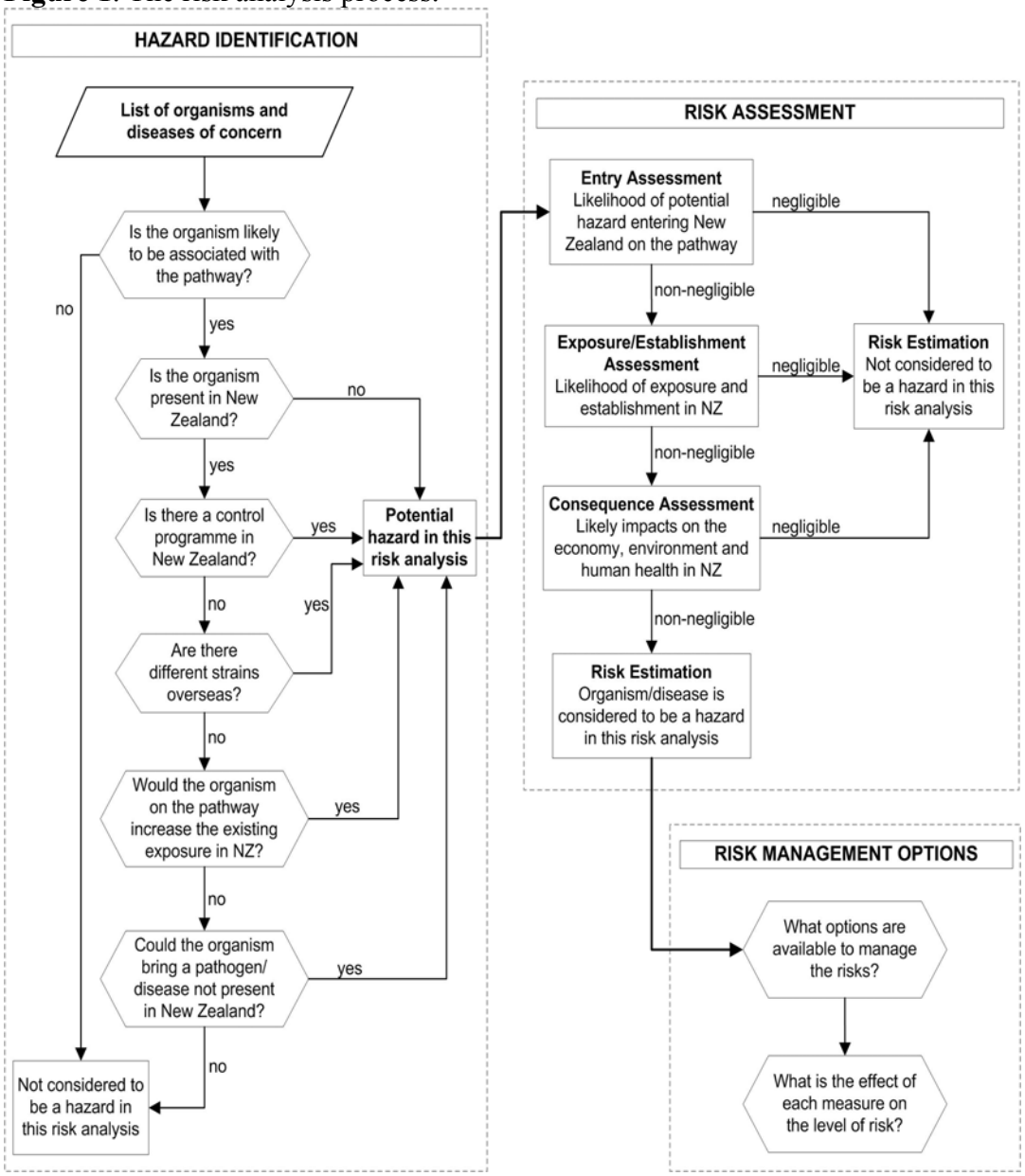
In addition, it is assumed that the germplasm is stored in the frozen state for at least 28 days before shipment to New Zealand, and that during this time the donor animals and all animals in contact with them will have remained healthy and free from any evidence of infection with disease agents that are assessed to pose a risk in this risk analysis.

4. Risk analysis methodology

The methodology used in this risk analysis is described in MAF New Zealand's *Risk Analysis Procedures Version 1* (MAF 2006) and is consistent with the guidelines in section 2 the *Terrestrial Animal Health Code* of the World Organisation for Animal Health in (OIE 2008a).

The risk analysis process used by MAFBNZ is summarised in Figure 1.

Figure 1. The risk analysis process.



4.1. PRELIMINARY HAZARD LIST

The hazard identification process begins with the collation of a list of organisms likely to be associated with the commodity. The basis for the preliminary hazard list is a list of all the hazards identified in MAF's *Import risk analysis*: *horses and horse semen* (MAF 2000a). This list was modified by eliminating organisms recognised in the risk analysis as being associated with a negligible risk in live horses. Protozoal parasites that cannot be transmitted by germplasm because they are transmitted by intermediate hosts (*Babesia equi*, *Babesia caballi* and *Trypanosoma* spp.) were also excluded.

It was not necessary to consider intestinal or internal protozoal parasites with complex lifecycles that are transmitted by the faecal-oral route (*Coccidia* spp. *Sarcocystis* spp.), or arthropod and helminth parasites (since these organisms are not transmitted in germplasm).

In response to comments made by reviewers of the risk analysis for horses and horse semen (MAF 2000b), West Nile virus was included in the preliminary hazard list. *Ehrlichia risticii* and *Ehrlichia equi* which are potential hazards for horses but not included in the 2000 risk analysis were included.

Equine herpesvirus-4 was not included in the MAF risk analysis for horses and semen (2000b) and occurs commonly in New Zealand. Therefore it is not included in Table 1.

The organisms of potential concern for equine germplasm and their occurrence in countries of concern are listed in Table 1.

Table 1. Organisms of potential concern

Hazard	Present in Australia?	Present in EU?	Present in North America?	Transmitted only by insect or helminth vectors♥	Preliminary hazard
Viruses					
African horse sickness virus	No*	No*	No*	Yes@	No
Vesicular stomatitis virus	No*	No*	Yes*	Yes@	No
Venezuelan equine encephalomyelitis virus	No*	No*	Not since 1971*	Yes@	No
Eastern equine encephalomyelitis virus	No*	No*	Yes*	Yes@	No
Western equine encephalomyelitis virus	No*	No*	Yes*	Yes@	No
Equine infectious anaemia virus	Yes*	Yes*	Yes*	Mechanical insect vectors also in semen	Yes
Equine influenza virus	No[1]	Yes*	Yes*	No@	Yes
Equine herpesvirus-1 (exotic strains)	Yes*	Yes*	Yes*	No@	Yes
Equine arteritis virus (exotic strains)	Yes?*	Yes*	Yes*	No@	Yes
Horse pox viruses	No*	No*	No*	No@	No
Japanese encephalitis virus	No*[2]	No*	No*	Yes@	No
West Nile virus	No**	Yes**	Yes*	Yes@	No
Rabies virus	No*	Yes*	Yes*	No@	Yes
Borna disease virus	No**	Yes**	No**	No@	Yes
Equine encephalosis	No**	No**	No@	Yes@	No

Hazard	Present in Australia?	Present in EU?	Present in North America?	Transmitted only by insect or helminth vectors♥	Preliminary hazard
virus					
Louping ill virus	No**	Yes**	No[3]	Yes@	No
Hendra virus	Yes**	No**	No**	No@	Yes
Getah virus	No**	No**	No**	Yes@	No
Bacteria					
Bacillus anthracis	Yes*	Yes*	Yes*	No@	Yes
Leptospira spp.	Yes*	Yes*	Yes*	No@	Yes
Taylorella equigenitalis	No*	Yes*	Yes*	No@	Yes
Taylorella asinigenitalis					
Burkholderia mallei	No*	No*	No*	No@	No
Burkholderia pseudomallei	Yes*	No*	No[4]	No@	Yes
Salmonella spp.	Yes*	Yes*	Yes*	No@	Yes
Other organisms					
Ehrlichia risticii and *Ehrlichia equi*	No**	Yes**	Yes	Yes@	No
Histoplasma capsulatum var. *farciminosum*	No*	No*	No*	No*	Yes#

Table footnotes:

* Country status as reported in the OIE databases Handistatus II (OIE 2008b) and /or World Animal Health Information Database (OIE 2008c).

** 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).

@ 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 5 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.

1. Australia is officially free following the eradication of the disease after a major epidemic (DAFF 2008).
2. Australia has occasionally reported cases in the far North but has not reported any cases to OIE in the last 3 years (OIE 2008c).
3. Swanepoel & Laurenson (2004).
4. CDC (2008).

Organisms considered to be preliminary hazards (marked "yes" in column 6 of Table 1) are submitted to *Risk analysis.*

4.2. RISK ANALYSIS

The steps in risk analysis are given in sections headed "Hazard identification", "Risk assessment", "Risk management" and "Risk communication".

4.2.1. Hazard identification

Each organism identified as a preliminary hazard (those marked "yes" in column 6 of Table 1) is subjected to hazard identification.

Hazard identification begins with a discussion on the relevant aspects of the epidemiology for that organism, as far as it is relevant to germplasm, in particular:

1. Whether the imported commodity could act as a vehicle for the introduction of the organism.
2. The occurrence of the organism in countries of relevance in this risk analysis.
3. If an organism is present in New Zealand whether:
    i. it is "under official control" in a pest management strategy as defined in the Biosecurity Act; or
    ii. whether more virulent strains are known to exist in other countries?

Organisms that are present in New Zealand are by definition not potential hazards unless either;

i) there is evidence that strains with higher pathogenicity than the endemic strains are likely to be present in the commodity to be imported, or
ii) the organism is under official control in New Zealand by means of a Pest Management Strategy under the Biosecurity Act (1993).

If the hazard identification process identifies the organism as a potential hazard it is subjected to risk assessment.

4.2.2. Risk assessment

The risk assessment procedure is summarised below:

Risk assessment

a)	Entry assessment -	the likelihood of the organism being imported in the commodity.
b)	Exposure assessment -	the likelihood of animals or humans in New Zealand being exposed to the potential hazard.
c)	Consequence assessment -	the consequences of entry, establishment or spread of the organism.
d)	Risk estimation -	a conclusion on the risk posed by the organism based on the entry, exposure and consequence assessments. If the risk estimate is non-negligible, then the organism is classified as a hazard.

If a risk assessment process leads to a conclusion that the organism under consideration is a hazard then this leads to the risk management step. However, if the conclusion is that the organism is not a hazard in the commodity, then there is no need to proceed to the risk management step.

Thus, it is important to note that not all of the above steps may be necessary in a risk assessment. The MAF Biosecurity New Zealand and OIE risk analysis methodologies make it clear that if there is a negligible likelihood of entry for a potential hazard, then the risk estimate is automatically negligible and the remaining steps of the risk assessment need not be carried out. The same situation arises where the likelihood of release is non-

negligible but the exposure assessment concludes that the likelihood of exposure to susceptible species in the importing country is negligible, or where both release and exposure are non-negligible but the consequences of introduction are concluded to be negligible.

4.2.3. Risk management

For each organism classified as a hazard, a risk management step is carried out, which identifies the options available for managing the risk. Where the *Code* lists recommendations for the management of a hazard, these are described alongside options of similar, lesser or greater stringency where available. In addition to the options presented, unrestricted entry or prohibition may also be considered for all hazards. Recommendations for the appropriate sanitary measures to achieve the effective management of risks are not made in this document. These will be determined when an import health standard (IHS) is drafted.

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

4.2.4. Risk communication

MAF releases draft import risk analyses for a six-week period of public consultation to verify the scientific basis of the risk assessment and to seek stakeholder comment on the risk management options presented. Stakeholders are also invited to present alternative risk management options that they consider necessary or preferable.

Following public consultation on the draft risk analysis, MAF produces a review of submissions and determines whether any changes need to be made to the draft risk analysis as a result of public consultation, in order to make it a final risk analysis.

Following this process of consultation and review, the Imports Standards team of MAF Biosecurity New Zealand decides on the appropriate combination of sanitary measures to ensure the effective management of identified risks. These are then presented in a draft IHS which is released for a six-week period of stakeholder consultation. Stakeholder submissions in relation to the draft IHS are reviewed before a final IHS is issued.

4.3. SPECIAL CONSIDERATIONS

Trade in germplasm may be preferred to trade in animals since it is cheaper and safer and it is able to deliver faster genetic improvement than by importing live breeding animals. Indeed, for almost 20 years it has been generally recognised, provided the code of practice published by the IETS is followed by the embryo transfer practitioners, in *vivo* derived embryo transfer is the safest way of exchanging genes (Thibier 2006).

Although equine embryo transfer was first reported to be successful in 1972, the technique was not implemented on a commercial scale until the early 1990s when it was developed in Argentina for use in polo pony mares. While breed societies have traditionally not allowed the registration of foals resulting from artificial breeding, there has recently been a relaxation of these rules which has increased the interest in this technology (Stout 2006). Nevertheless, the cost of embryo transfer in horses is still very high relative to the cost of the same technologies in other species. For example, in the USA embryo transfer has been assessed to be justifiable only if the value of the newborn foal was greater than $10,000 (US) as the cost of the technology was from $3,000 (US) to $6,000 (US) per pregnancy (McCue and Troedsson 2003).

The high cost of equine embryo transfer has meant that there has been relatively little incentive to develop the technology on a commercial scale, and as a result, there are very few studies that have examined the biosecurity risks associated with *in vivo* equine embryos. Therefore, this risk analysis will also draw upon the results of studies undertaken in production animal species that have examined closely related pathogens.

However, where no relevant data relating to the safety of embryos for a particular disease exists, it will be assumed that, if both the male and female donors from which the embryos are derived meet the disease status requirements that would allow them to be imported into New Zealand, the embryos may be imported without further restrictions.

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5. Equine infectious anaemia virus

5.1. HAZARD IDENTIFICATION

5.1.1. Aetiological agent

Family: Retroviridae, Genus: *Lentivirus*, Species *Equine infectious anaemia virus* (EIAV) (Linial et al 2005).

5.1.2. OIE list

Listed.

5.1.3. New Zealand status

EIAV is an unwanted notifiable organism (MAF 2008).

5.1.4. Epidemiology

EIAV has a worldwide distribution (Issel and Foil 1984). Horses and donkeys and probably zebras are susceptible. Virus is spread mechanically by large biting flies e.g. *Stomoxys calcitrans* (stable fly), *Chrysops* sp. (deer fly), and *Tabanus* sp. (horse fly) (Foil et al 1987; Foil et al 1983).

Morbidity is highly variable and depends upon the dose of virus delivered by the insect vector. Infected individuals can become acutely ill and die but the course of the disease is usually short and mild. Acute infection is characterised by fever loss of appetite, anaemia and jaundice and is often followed by recurrent febrile episodes. Chronically infected horses show mild clinical signs. The incubation period is usually 1-3 weeks but may be several months. Antibodies develop 1-2 weeks after infection (DEFRA 2006). Infected horses become persistently infected and act as a source of virus (Cook and Issel 2004; Radostits et al 2007).

The disease is generally diagnosed by serological tests such as the agar gel immunodiffusion test (AGID) or an ELISA. The AGID is the OIE prescribed test, and as discussed in the *Manual,* for 2-3 weeks after infection horses will usually give negative serological reactions. In rare cases the post-infection time prior to the appearance of detectable antibody may extend up to 60 days when using the AGID test (Ostlund 2008).

Intrauterine infection has been recognised and may result in either abortion or the birth of infected foals. Mares can be infected following artificial insemination with infected semen (CFIA 2006; Radostits et al 2007).

5.1.5. Hazard identification conclusion

Intrauterine infection with EIAV is possible and although there is uncertainty surrounding the risk posed by embryos, it is accepted that the disease can be transmitted by artificial insemination. Therefore the organism is assessed to be a potential hazard in the commodities.

5.2. RISK ASSESSMENT

5.2.1. Entry assessment

EIAV is present in all of the countries that are considered in this risk analysis, and it is recognised that infected animals may become lifelong carriers. There is clear evidence that the virus can be transmitted by semen (CFIA 2006; Radostits et al 2007).

Although there are no published investigations on EIAV transmission by equine embryos, there are a number of studies on closely related lentiviruses in other species. Studies on caprine arthritis encephalitis virus (CAEV) (Ali Al Ahmad et al 2006; Lamara et al 2002; Wolfe et al 1987), bovine immunodeficiency virus (Bielanski et al 2001) and maedi visna virus (MVV) (Dawson and Wilmut 1988; Vainas et al 2006; Woodall et al 1993) have shown that zona-pellucida-intact embryos that are prepared according to IETS specifications, including trypsinisation, are unlikely to transmit lentiviruses.

As a result, IETS has classified CAEV as a Category 3 organism which means that "preliminary evidence indicates that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer according to the IETS manual, but for which additional *in vitro* and *in vivo* experimental data are required to substantiate the preliminary findings" (IETS 2004).

However, IETS has classified MVV in Category 4 agent which is an organism for which studies have been done, or are in progress that indicate either that no conclusions are yet possible with regard to the level of transmission risk, or that the risk of transmission via embryo transfer might not be negligible even if the embryos are properly handled according to the IETS Manual.

Based on the available evidence of transmission in semen and the uncertainty regarding embryos, the likelihood of entry of EIAV in the commodities is considered to be non-negligible.

5.2.2. Exposure assessment

Imported embryos or semen would be transplanted or inseminated into naïve New Zealand mares. Therefore the likelihood of exposure is non-negligible.

5.2.3. Consequence assessment

EIA virus affects only horses, donkeys and zebras. As it is not a zoonotic disease there would be no consequences for humans.

Direct consequences would in the first instance be confined to the recipient females that were inseminated or implanted with the imported genetic material. If the disease were to become established in recipient animals and then spread to other animal populations, then the direct consequences would be more broadly felt. Since infected animals may become chronic carriers of the virus, and since the biting fly *Stomoxys calcitrans* is present in many parts of New Zealand, it is possible that infection may spread to other equine populations, including commercial breeders, horses used for recreation and small feral populations. However, there would be no effect on native animals. As such, the direct consequences would be those in relation to animal disease in various equine populations, including a low

level of mortality, and more widespread sickness requiring treatment and causing poor performance.

Indirect consequences could arise if a control or eradication programme was initiated, and depending on what control measures were instituted, there could be some disruption to various equine events. However, since EIA is a disease of considerable international concern, by far the most important indirect consequences would be the international trade effects, as several countries require certification of freedom from infection for horses that are exported from New Zealand and many countries would impose costly conditions on exports of horses from this country.

Therefore the consequences of introduction are considered to be non-negligible.

5.2.4. Risk estimation

Since entry, exposure and consequence assessments are all non-negligible, the risk is assessed as non-negligible and EIAV is classified as a hazard in the commodity. Therefore, risk management measures can be justified.

5.3. RISK MANAGEMENT

5.3.1. Options

The following points should be considered when drafting options for the efficient management of EIAV in the commodity.

- Infected animals may be life-long carriers of virus.
- In rare cases the prescribed test for international trade (AGID) may require up to 60 days from infection to elapse before antibody is detectable.
- In most cases, antibody can be detected within 45 days of infection using the AGID (Cook & Issel 2004).
- Semen of infected animals may be contaminated with virus.
- The likelihood that embryos from infected animals would be infected with EIAV is non-negligible.

The *Code* chapter relating to the importation of horses for EIA is:

Article 12.6.2.

Recommendations for the importation of equines

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 3 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

The *Code* does not make recommendations relating to germplasm.

The following options given in ascending order of stringency could be considered for effective management of the risk of EIAV in the commodities:

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.

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, this 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.

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6. Equine influenza virus

6.1. HAZARD IDENTIFICATION

6.1.1. Aetiological agent

Family: *Orthomyxoviridae*; Genus: *Influenzavirus*; Species: *Influenza A* (Kawaoka et al 2005).

Equine influenza (EI) is caused by subtypes H7N7 and H3N8 which were previously designated equine 1 and equine 2 viruses respectively.

6.1.2. OIE list

Listed.

6.1.3. New Zealand status

Influenzavirus type A (exotic equine strains) are listed as unwanted exotic organisms (MAF 2008).

6.1.4. Epidemiology

EI is a contagious respiratory disease of horses, donkeys, mules and zebras (Newton and Mumford 2004). It has a global distribution and, until recently, the only large horse populations assessed free from this disease were in Australia and New Zealand (Radostits et al 2007). However, EI was diagnosed in Sydney on 22 August 2007 and spread rapidly to infect large parts of New South Wales and southern Queensland. After an extensive eradication campaign the Australian authorities have declared the previously infected states to be officially free from EI (DAFF 2008). All other countries of relevance to this risk analysis are endemically infected with EI.

The virus has only been isolated from respiratory tissues and discharges. As highlighted previously (MAF 2000), no reports have been found that have reported the transmission of EI infection in semen or by artificial insemination. No studies have been identified that have examined the potential for EI virus to be transmitted by embryos. No reports were found of the transmission of other Orthomyxoviridae by embryos in other animal species. Horses that recover from EI do not remain carriers of the virus. Therefore, the likelihood that embryos derived from healthy horses that have remained healthy for 28 days after embryo collection would be infected with EI virus is negligible.

Article 12.7.4. of the *Code* states that *in vivo* derived equine embryos should not be subject to sanitary measures for EI.

6.1.5. Hazard identification conclusion

There is no evidence that EI is transmitted by equine embryos or semen and, consistent with the advice of the OIE, this virus is not assessed to be a potential hazard in the commodities.

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7. Equine herpesvirus-1

7.1. HAZARD IDENTIFICATION

7.1.1. Aetiological agent

Family: *Herpesviridae*; Subfamily: Alphaherpesvirinae; Genus: *Varicellovirus*; Species: *Equid herpesvirus* 1 (EHV-1) (Davison et al 2005).

EHV-1 viruses causing equine abortions are grouped into two strains, EHV-1B and EHV-1P based on DNA restriction enzyme fragment analysis (Crabb and Studdert 1996).

7.1.2. OIE list

"Equine rhinopneumonitis" is listed in the *Code* as a disease of horses, and there is a chapter on this disease. The 2008 *Manual of Diagnostic Tests* explains that "equine rhinopneumonitis" is a collective term for any one of several highly contagious clinical disease entities of equine animals that may occur as a result of infection by either of two closely related herpesviruses, EHV-1 and EHV-4.

7.1.3. New Zealand status

There have been no recorded cases of EHV-1B (MAF 2000). EHV-1B is therefore considered exotic but EHV-1P and EHV-4 are present in New Zealand.

7.1.4. Epidemiology

EHV-1 occurs world-wide and causes abortions and sometimes respiratory or neurological disease. It is most importantly considered to cause abortions.

Horses infected with EHV-1 are likely to become long term latent carriers of the virus, showing no signs of infection. Latently infected horses would not excrete the virus except when it is reactivated due to stress or steroid treatment. Carriers of virus are likely to be serologically positive but no distinction can be made between EHV-1B and EHV-1P by serological tests suitable for export/import testing.

The virus can be excreted in semen (Temple et al 1996). PCR has been used for the detection of EHV-1 in semen (Carvalho et al 2000) and real time PCR is a well established method for detection of the closely related *Bovine herpesvirus* 1 (BHV-1) in bovine semen (Carvalho et al 2000; Wang et al 2007; Wang et al 2008).

A Brazilian study detected EHV-1 in an equine embryo by PCR but not by virus isolation. However, this study was unable to rule out contamination due to endothelial or trophoblastic cells aggregated in uterine flushing. This suggests that, the sample may not have been subjected to washing as recommended by IETS (Carvalho et al 2000).

Studies by Hebia et al have been summarised in a research update published by IETS (IETS 2007). These studies showed that EHV-1 can adhere to the zona pellucida of embryos and could not be removed by washing. However, the virus could be removed by trypsin treatment. These findings are similar to those reported in several studies done on

herpesviruses of other animal species (Carvalho et al 2000; Singh 1983; Singh et al 1982; Stringfellow et al 1990; Thibier and Nibart 1987; Wang et al 2007; Wang et al 2008).

Both zebras and donkeys can be infected by herpes viruses but these differ from the equivalent viruses found in horses (Borchers et al 2005; Browning and Agius 1996; Browning et al 1988; Kleiboeker et al 2002).

7.1.5. Hazard identification conclusion

EHV-1B is concluded to be exotic to New Zealand. Since EHV-1 is removed from embryos by trypsin treatment, EHV-1 is not a potential hazard in embryos that meet the commodity definition.

EHV-1 virus can be excreted in semen. Therefore, EHV-1B strains of the virus are assessed to be potential hazards in semen.

7.2. RISK ASSESSMENT

7.2.1. Entry assessment

Exotic EHV strains are likely to occur in all countries with which this risk analysis is concerned. Horses could be in the incubation or acute stage of infection or latent carriers of the virus, when used as semen donors. Embryos that meet the commodity definition pose negligible risk. However, since the virus can be excreted in semen the likelihood of entry in imported semen is non-negligible.

7.2.2. Exposure assessment

Imported semen will be used to inseminate mares. Therefore, the likelihood that naïve mares would be exposed to infection is non-negligible.

7.2.3. Consequence assessment

Equine herpes viruses affect only horses, donkeys and zebras. Since there are different viruses in each of these species, it is unlikely that there would be crossover of infections from one equine species to another. As it is not a zoonotic disease there would be no consequences for humans.

Infection by either EHV-1 or EHV-4 is characterised by a primary respiratory tract disease of varying severity that is related to age and immunological status of the infected animal. Infections of EHV-1 in particular are capable of progression beyond the respiratory mucosa to cause more serious disease manifestations of abortion, perinatal foal death, or neurological dysfunction.

Direct consequences would in the first instance be confined to the recipient females that were inseminated or implanted with the imported genetic material. EHV is most importantly considered to cause abortions. Therefore financial losses for breeders may result if there were outbreaks of abortion. However, as infection with EHV-4 and EHV-1P is already widespread in New Zealand, there may be sufficient population immunity to make outbreaks of abortion rare even in directly exposed populations.

If an exotic EHV infection were to become established in recipient animals and then spread to other animal populations, then the direct consequences may be more broadly felt. Since infected animals may become chronic carriers of the virus, it is possible that infection may spread over time to other equine populations, including commercial breeders, horses used for recreation and small feral populations.

However, there would be no effect on native animals. As such, the direct consequences would be those in relation to animal disease in various equine populations, including mild respiratory disease, losses due to abortions and perinatal foal death, and rare cases of neurological disease.

The indirect consequences of EHV would include the inability to use sick horses normally for riding, training or racing. In addition, depending on what level of control measures were instituted, there could be some disruption to various equine events and if vaccination became necessary that would impose extra costs on animal owners. There may be a range of international trade reactions from trading partners, many of whom currently require certification of establishment freedom from EHV-1.

Therefore the consequences of introduction are considered to be non-negligible.

7.2.4. Risk estimation

Since entry, exposure and consequence assessments for the introduction of exotic strains are all non-negligible, the risk is assessed to be non-negligible and EHV-1B is classified as a hazard in semen. Therefore, risk management measures for semen can be justified.

7.3. RISK MANAGEMENT

7.3.1. Options

The following points should be considered when drafting options for the efficient management of EHV-1B in semen:

- Donor stallions that do not have antibody against EHV-1 would be free from infection. However, since the virus is so common and cross reactions occur with EHV-4 infected horses it would be extremely difficult to find such horses. Any restrictions based on negative serology would be exceedingly trade restrictive and would probably be rightly resisted by the horse industries.
- Importations could be restricted to semen from donors that are not housed in premises in which active outbreaks of the disease are occurring.
- Semen could be tested by PCR for the presence of EHV-1 virus.
- Demonstration of EHV-1 virus in semen could be followed by isolation and identification of the strain of virus. Semen infected with EHV-1B could be disqualified. However testing for virulence would not be practical.

The *Code* recommendations for EHV-1 are given below.

Article 12.9.2.

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, 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 was reported during that period.

The following options given in ascending order of stringency could be considered for effective management of the risk of EHV-1B in semen:

Option 1.

Semen could be imported provided that donor stallions:

i. were kept for the 21 days prior to semen collection in an establishment where no case of equine herpes virus type 1 infection was reported during that period; and
ii. showed no clinical sign 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 of the virus.

Option 2.

Semen could be imported provided each batch of semen was tested by a PCR test for 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.

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8. Equine arteritis virus

8.1. HAZARD IDENTIFICATION

8.1.1. Aetiological agent

Family: *Arterivirdae*; Genus: *Arterivirus*; Species: *Equine arteritis virus*. North American and European strains are described (Snijder et al 2005).

Asinine strains of the virus probably differ from equine strains (Paweska 1997a; Paweska 1997b; Stadejek et al 2006).

8.1.2. OIE list

Listed.

8.1.3. New Zealand status

Equine arteritis virus (EAV) is listed as an unwanted notifiable organism (MAF 2008). There is a control programme for the disease administered by the New Zealand Equine Health Association. At the end of 2006 there was only one known shedder stallion in New Zealand (O'Flaherty and Reid 2007). The EAV strains present in Australia and New Zealand do not cause disease. In contrast, a wide range of clinical signs are associated with infection with the strains present in the other countries covered in this risk analysis.

8.1.4. Epidemiology

Equine viral arteritis (EVA) occurs world-wide. The virus infects horses and donkeys (Timoney and McCollum 2004), but there is no information about its occurrence in zebras. The virus is transmitted predominantly by the respiratory route by horses in the acute phase of the disease but is also transmitted venereally by shedder stallions (Timoney and McCollum 2004). Recently infected horses shed the virus in the respiratory tract for 7-16 days. Recovered serologically positive animals are solidly immune and are not carriers of the virus, except in the case of stallions that shed the virus in their semen for protracted periods. There is some evidence that infected mares may harbour infection in the genital tract (Timoney and McCollum 2004).

Infection with the virus is often subclinical and the disease has never been seen in New Zealand. However, a wide range of signs have been associated with the disease including fever, respiratory signs, abortion, oedema of the extremities, ataxia and paresis in other countries (Timoney and McCollum 2004).

Available vaccines are highly effective and immunised animals are solidly immune for at least several years. A modified live virus vaccine induces a solid immunity after a single vaccination. An inactivated vaccine is less potent and booster doses are required to induce a solid immunity, but it can be safely used in pregnant mares (Timoney and McCollum 2004). Immunization of naïve stallions and immature colts between 6 and 12 months of age prevents establishment of the carrier state (Timoney and McCollum 2004). Stallions that are serologically negative or have been vaccinated at an appropriate age and are serologically positive are not carriers of the virus.

Asinine strains of EAV virus appear to differ from equine strain (Paweska 1997a; Paweska 1997b; Stadejek et al 2006). There is no information about the occurrence of the virus in zebras but wild zebras in South Africa did not have antibody against the virus (Barnard 1998). However, it is assumed that zebras could be infected with either equine or asinine strains of EAV.

8.1.5. Hazard identification conclusion

Since EAV can be excreted in the semen of carrier stallions and there is some evidence that suggests that embryos could transmit the virus it is assessed to be a potential hazard in the commodity.

8.2. RISK ASSESSMENT

8.2.1. Entry assessment

Since the virus is endemic in Europe and North America, germplasm donors could be infected. While transmission in semen of carrier stallions is well accepted, there is less evidence to suggest that transmission is possible via embryos (Timoney and McCollum, 2004).

Several studies on porcine reproductive and respiratory syndrome virus, which is a closely related arterivirus, indicate that it is not transmitted by embryos (Prieto et al 1996; Randall et al 1999; Smits et al 2001; Smits et al 2002; Torremorell et al 2000). However, a research update published by IETS contains summaries of investigations that show that ovarian tissues and follicular fluid from infected mares can contain EAV and that it could not be removed from embryos by standard washing procedures (IETS 2007).

Since semen or embryos of infected donors may harbour the virus the likelihood of entry is assessed to be non-negligible.

8.2.2. Exposure assessment

Imported semen or embryos would be inseminated or transplanted into naïve New Zealand mares. Therefore, the likelihood of exposure is non-negligible.

8.2.3. Consequence assessment

Equine arteritis virus affects only horses, donkeys and possibly zebras. Since the viruses of horses and donkeys are known to be different, infection would not be expected to cross between equine species. As it is not a zoonotic disease there would be no consequences for humans.

The majority of naturally acquired infections with EAV are subclinical. While the strains present in this country do not produce clinical signs, exotic strains may result in fever, depression, anorexia, dependent oedema, especially of the limbs, scrotum and prepuce in the stallion, conjunctivitis, an urticarial-type skin reaction, abortion and, rarely, a fulminating pneumonia or pneumo-enteritis in young foals. However, apart from mortality in young foals, the case fatality rate in outbreaks of EVA is very low. Affected horses almost invariably make complete clinical recoveries.

Direct consequences of EAV infection would depend on the pathogenicity of the particular exotic strain introduced. Clinical disease would in the first instance be confined to the recipient females that were inseminated or implanted with the imported genetic material. Spread from the index case would occur readily through contact by the respiratory route. Since infected animals may become chronic carriers of the virus, it is possible that infection may spread to other equine populations, including commercial breeders, horses used for recreation and small feral populations.

However, there would be no effect on native animals. As such, the direct consequences would be those in relation to animal disease in various equine populations.

Indirect consequences would include those related to treatment of sick animals and the inability to use affected animals for recreational purposes as well as training or racing. Moreover, depending on what control measures were instituted in the event of a widespread outbreak, there could be disruption to various equine events.

Even a limited outbreak caused by the introduction of an exotic strain would seriously affect the export of horses, particularly to Australia but also to a wide range of other countries.

Therefore the consequences of introduction are considered to be non-negligible.

8.2.4. Risk estimation

Since entry, exposure and consequence assessments are all non-negligible, the risk is assessed to be non-negligible and EAV is classified as a hazard in the commodities. Therefore, risk management measures can be justified.

8.3. RISK MANAGEMENT

8.3.1. Options

The following points should be considered when drafting options for the efficient management of EAV in the commodities:

- Stallions may be carriers of EAV and shed the virus in their semen.
- Mares recover spontaneously from infection and do not remain carriers.
- Serologically positive mares are not carriers.
- Vaccination is highly effective when used according to manufacturer's instructions in horses that are not already infected.
- The virus infects horses and donkeys and although information is lacking about zebras it should be assumed that they could be infected.
- There is no evidence to suggest that the related arterivirus, PRRSV, is transmitted by embryos.
- There is little useful information about the transmission of the virus by embryos; therefore both male and female donors of the embryos should be free from infection.

The *Code* recommendations for the importation of semen are:

Article 12.10.4.

Veterinary Authorities of *importing countries* should require:

for semen

the presentation of an *international veterinary certificate* attesting that the animal donors:

1. were kept for the 28 days prior to semen collection in an *establishment* where no equine has shown any clinical sign of EVA during that period;
2. showed no clinical sign of EVA on the day of semen collection;
3. were subjected between 6 and 9 months of age to a test for EVA as prescribed in the *Terrestrial Manual* on a blood sample with stable or decreasing titre, immediately vaccinated for EVA and regularly revaccinated according to the manufacturer's instructions; or
4. were subjected to a test for EVA as prescribed in the *Terrestrial Manual* on a blood sample with negative results, immediately vaccinated for EVA, kept for 21 days following vaccination separated from other equidae and regularly revaccinated according to the manufacturer's instructions; or
5. were subjected to a test for EVA as prescribed in the *Terrestrial Manual* on a blood sample with negative results within 14 days prior to semen collection, and had been separated from other equidae from the time of the taking of the blood sample to the time of semen collection; or
6. have been subjected to a test for EVA as prescribed in the *Terrestrial Manual* on a blood sample with positive results and then: either
    a. were subsequently test mated to two mares within 12 months prior to semen collection, which were subjected to two tests for EVA as prescribed in the *Terrestrial 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 *Terrestrial Manual* with negative results, carried out on semen collected within one year prior to collection of the semen to be exported.

There are no recommendations relating to embryos in the *Code*.

Options for the effective management of EAV in the commodities, in ascending order of stringency are:

For importation of embryos

Option 1.

Embryos could be imported provided that:

i. male donors meet the requirements of Article 12.10.4 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 of Article 12.10.4 of the *Code*; and
ii female donors of ova 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.

For importation of 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.

Alternatively, provided an assessment of EVA controls in an exporting country can demonstrate a level of protection that is equivalent to New Zealand's, then importing infectious semen from shedder stallions may be considered. Permission from the Chief Technical Officer to receive infectious semen would be required.

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9. Borna disease virus

9.1. HAZARD IDENTIFICATION

9.1.1. Aetiological agent

Family: *Bornaviridae*; Genus: *Bornavirus*; Species: *Borna disease virus*. It is the only member of its family.

9.1.2. OIE list

Not listed.

9.1.3. New Zealand status

Listed on the unwanted organisms register as an exotic, unwanted organism (MAF 2008).

9.1.4. Epidemiology

Borna disease is typically a disease of horses but sheep and a variety of other animals including goats, deer, rabbits (Rott et al 2004), lynx (Desgiorgis et al 2000), and foxes may be infected (Dauphin et al 2001). The disease occurs most commonly in Germany and Switzerland where it is endemic (MAF 2000). However, serologically positive animals have also been found in Poland, the Netherlands, Switzerland, Iran (Rott et al 2004), and Japan (Hagiwara et al 1997; Hagiwara et al 1996; Hagiwara et al 2002; Inoue et al 2002; Nakamura et al 1996; Nakamura et al 1995; Okamoto et al 2002) and Borna virus RNA has recently been found in France (Dauphin et al 2001; Dauphin and Zientara 2003) and Finland (Kinnunen et al 2007). Reports on the demonstration of antibodies in horses have also come from North America (Kao et al 1993) and Israel (Teplitsky et al 2003). The virus has been demonstrated in cats in the UK (Reeves et al 1998). Several authors have suggested that it is an emerging disease and that many species of animals may be infected (Boucher et al 1999; Ludwig and Bode 2000). However, a recent study found no evidence of the virus in Australia (Kamhieh et al 2006). Since it is not an OIE listed disease, information is not available on OIE databases.

A closely related virus has been found in mallards and jackdaws in Sweden (Berg et al 2001).

In sheep and horses, Borna disease typically presents as a disease of the nervous system, but infection is most commonly subclinical (Rott et al 2004). Antibody to the virus has been found in humans suffering from psychosomatic disorders (Bode et al 1996; Rott et al 1985). However, the exact role of the virus in human infections and as a cause of psychosomatic disorders remains controversial. The specificity of demonstrated antibody and the accuracy and reliability of the PCR test to demonstrate the presence of viral RNA has been questioned, and the issues remain unresolved (Carbone 2001; Staeheli et al 2000). Some recent studies have concluded that the virus infects humans (Chalmers et al 2005; Kishi et al 1995) while others have yielded contrary evidence (Thomas et al 2005; Wolff et al 2006).

The incubation period is thought to vary from 4 weeks to several months (Ludwig and Kao 1990). In mice the disease enters the body through the olfactory epithelium and migrates along nerve axons to the brain (Carbone et al 1987; Morales et al 1988; Sauder and Staeheli 2003). The virus can be experimentally transmitted to rats by inoculation into the footpads. However, neurectomy prevents the disease occurring, thus demonstrating that transfer of the virus to the brain is by the intra-axonal route (Carbone et al 1987). It is excreted in nasal secretions, saliva and urine (Rott et al 2004; Vahlenkamp et al 2002). In an experimental situation the disease was transmitted from persistently infected rats to naïve rats via the olfactory route. This has led to the suggestion that rats could be a source of infection for farm animals (Sauder and Staeheli 2003). Shrews have recently been suggested as natural hosts for the virus (Hilbe et al 2006). Vertical transmission has not been reported. Most infections are thought to be subclinical (Ludwig and Kao 1990) and the virus persists in carriers for at least 2 years, as demonstrated by the presence of viral RNA in peripheral mononuclear cells (Vahlenkamp et al 2002). Viral RNA has been demonstrated in the peripheral mononuclear cells of cattle (Hagiwara et al 1996), sheep (Hagiwara et al 1997; Vahlenkamp et al 2000; Vahlenkamp et al 2002), horses (Nakamura et al 1995; Vahlenkamp et al 2002), cats (Nakamura et al 1996; Reeves et al 1998) and humans (Kishi et al 1995; Vahlenkamp et al 2000). Natural transmission is presumed to occur via direct contact, fomites and food, inhalation, and ingestion (Rott et al 2004).

Despite the fact that Borna disease has been known for more than 250 years (Rott et al 2004), knowledge about the disease is still fragmentary and incomplete. The interpretation of the results of diagnostic tests is problematical. Although viral RNA has been demonstrated in an increasing number of countries and animal species, the occurrence of the disease is still mainly confined to parts of Germany and surrounding countries. One report suggests that the results reported by workers using RT- nested PCR are highly controversial and should be interpreted with caution as they could be due to accidental sample contamination (Staeheli et al 2000). Studies using RT-PCR have not generally been confirmed by viral isolation.

The disease is not regarded by OIE as important to trade and it only occurs sporadically in countries where it does occur. However, in Germany it is notifiable and is controlled by a slaughter-out policy (Rott and Herzog 1994).

Borna disease can be diagnosed by demonstration of typical lesions by histopathology, and demonstration of virus by *in situ* hybridisation or RT-PCR, virus isolation or by the intracerebral inoculation of rabbits. Serological methods are also available. Since there is a level of controversy associated with sensitivity, specificity, or accuracy of most testing methods, there are no tests, which are validated and suitable for testing live animals for international trade purposes.

There is no evidence that the virus is transmitted in semen or embryos but since it is associated with peripheral mononuclear cells it could be excreted in semen in some circumstances.

The disease occurs naturally in donkeys (Kolodziejek et al 2005) and although no reports were found of the disease in zebras, it is assumed it could occur in all equidae.

9.1.5. Hazard identification conclusion

Borna disease is regarded as an exotic disease. It occurs in some European countries and the virus may occur in several countries where the disease has not been, or rarely been

described. No evidence was found relating to the potential for the virus to be transmitted in germplasm. Therefore, the virus is assessed to be a potential hazard in the commodities.

9.2. RISK ASSESSMENT

9.2.1. Entry assessment

9.2.1.1. Semen

There is nothing in the literature that indicates that Borna disease is spread venereally. There is no definite information about the potential for the virus to contaminate semen. Much of the available information on the disease is based on studies in rats. In rats infected as adults, the virus multiplies only in neurons. However, in rats infected as neonates, the virus is found in all organs and these animals remain persistent shedders of virus. Virus can be shed in various body secretions, including nasal secretions, faeces, and urine. It is not known to what extent the pathogenesis in horses parallels that of rats.

Peripheral mononuclear cells of horses, cattle, sheep, cats and humans have been found to contain viral RNA (Hagiwara et al 1997; Hagiwara et al 2002; Schindler et al 2007; Vahlenkamp et al 2000; Vahlenkamp et al 2002; Wensman et al 2007). However, the validity of the test methods has been questioned (Staeheli et al 2000).

The likelihood that semen could be contaminated with infected mononuclear cells cannot be ignored since concomitant bacterial infections could result in leakage of mononuclear cells that could be contaminated with virus, into semen. In addition, contamination of semen by urine could introduce the virus.

In the absence of definitive information to the contrary, the likelihood of the entry of virus in semen is assessed to be low.

9.2.1.2. Embryos

No information was found about embryos derived from Borna disease infected animals. However, since the virus is excreted in urine, saliva and nasal secretions, and viral RNA is found in mononuclear cells, a conservative approach suggests that the likelihood of entry in embryos should be assessed to be low.

9.2.2. Exposure assessment

Imported embryos and semen would be inseminated/transplanted into susceptible recipients in New Zealand. Natural transmission of the virus probably occurs through the olfactory route and is transported intra-axonally to the brain. It is not known whether infection through the uterus is possible. In view of this uncertainty, the likelihood of exposure is assessed to non-negligible.

9.2.3. Consequence assessment

Although there has been some suggestion of a link between Borna disease virus infection and the occurrence of psychosomatic diseases in humans (Bode et al 1996; Rott et al 1985), the nature of the association is unclear. However, for the purposes of this risk analysis it is concluded that the uncertainty justifies a non-negligible assessment for human health.

Most Borna disease virus infections of horses are asymptomatic, but when clinical disease is seen it usually manifests as nervous signs and almost invariably ends fatally (Ludwig and Kao 1990). Borna disease also occurs naturally in donkeys (Kolodziejek et al 2005) and although no reports were found of the disease in zebras, it is assumed it is possible. If the virus were introduced in imported semen and embryos, the direct consequences would in the first instance be confined to the recipient females that were inseminated or implanted with the imported genetic material. If the disease were to become established in recipient animals and then spread to other animal populations, then the direct consequences would be more broadly felt.

Natural transmission is presumed to occur via direct contact, fomites and food, inhalation, and ingestion. Since the virus is known to infect a wide variety of mammals (Dauphin and Zientara 2003; Desgiorgis et al 2000; Rott et al 2004) and birds (Berg et al 2001) the introduction of Borna disease virus could potentially result in the establishment of an economically important disease of horses, cattle, sheep and birds. Since nervous disease has been reported in ostriches caused by Borna disease virus (Ashash et al 1996) it is possible that kiwi may be similarly susceptible.

Therefore the consequences are assessed to be low but non-negligible.

9.2.4. Risk estimation

Because entry, exposure and consequence assessments are non-negligible, the risk estimate for Borna disease virus is assessed to be non-negligible and it is classified as a hazard in the commodities. Therefore, risk management measures can be justified.

9.3. RISK MANAGEMENT

9.3.1. Options

The following points should be considered when drafting options for the efficient management of Borna disease virus in the commodities:

- No validated methods are available for diagnosis in live animals. Non-validated methods include virus isolation, serology and RT-PCR, but interpretation of results is controversial.
- Occurrence of disease in horses is essentially still confined to Germany and surrounding countries.
- The disease is notifiable in Germany.
- Some authorities consider it to be an emerging disease, possibly with a worldwide distribution.

Since Borna disease is not listed by the OIE, no international standards for risk management exist.

Options for the effective management of the risk posed by Borna disease virus in the commodities, in ascending order of stringency, include:

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.

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10. Hendra virus

10.1. HAZARD IDENTIFICATION

10.1.1. Aetiological agent

Family: *Paramyxoviridae*; Subfamily: *Paramyxovirinae*; Genus: *Henipavirus*; Species *Hendra virus* (Lamb et al 2005).

10.1.2. OIE list

Not listed.

10.1.3. New Zealand status

Classified as a notifiable organism (MAF 2008).

10.1.4. Epidemiology

The virus only has only been reported in Australia where it causes a rare, deadly disease of horses and humans. Between 1994 and the time of writing this report the disease has been recorded in 34 horses including one outbreak involving 20 horses. In the last 14 years at least six people have been infected and three died from the infection (Anonymous 2008).

The incubation period is up to 14 days. Signs of infection include fever and neurological signs.

Information reviewed by Williamson (2004) indicates that cats and guinea pigs have been infected experimentally. The disease is not highly contagious and spread from horses to humans or to other horses only seems to have occurred following close contact and even contact with body fluids and organs during post mortem examination of dead horses. In experiments transmission from animal to animal has not occurred except in one case from a cat to a horse.

The natural source of infection is the fruit bat (*Pteropodidae*). The prevalence of antibodies in wild caught *Pteroptus* spp. was 42% (Mackenzie 1999).

There is no evidence that the virus can establish naturally as a self sustaining infection of horses or animals other than fruit bats. Sporadic cases are likely to occur from accidental transmission from fruit bats in Australia. It is not known if the virus can be carried by recovered horses. However, in up to 10% of human cases that recovered from infection with the closely related Nipah virus, subsequent reactivation of the virus occurred. A horse that recovered from the infection in Australia was euthanased (Glanville 2008).

There is no evidence that the virus can be transmitted in germplasm.

10.1.5. Hazard identification conclusion

Hendra virus is unlikely to establish in New Zealand in the absence of fruit bats. It is a rare disease in Australia. Horses showing clinical signs of Hendra virus infection would not be

used as germplasm donors and, if germplasm was collected from animals in the incubation period, they would develop clinical signs during the 28 days that semen was held before shipment. There is no evidence that Hendra virus can be transmitted in germplasm. The virus is unlikely to be able to establish in a country where fruit bats do not occur. Therefore, Hendra virus is not assessed to be a potential hazard in the germplasm.

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11. Rabies virus

11.1. HAZARD IDENTIFICATION

11.1.1. Aetiological agent

Family: *Rhabdoviridae*; Genus: *Lyssavirus*; Species: *Rabies virus* (Tordo et al 2005).

11.1.2. OIE list

Listed.

11.1.3. New Zealand status

Rabies virus is listed as an unwanted, notifiable organism (MAF 2008).

11.1.4. Epidemiology

Rabies is a disease of all mammals including equidae and humans. It is characterised by severe nervous signs and is invariably fatal.

Rabies occurs widely around the world but there are a number of countries, including mainly island and peninsular countries, that are free from the disease. In some countries such as Denmark and Australia that are free from true rabies virus, bats are endemically infected with a closely related lyssaviruses (Swanepoel 2004).

In all endemically infected countries rabies virus is maintained in a population of domestic or wild carnivores or bats. True rabies in bats is confined to the Americas (Swanepoel 2004) but infections of bats with related lyssaviruses occur in Europe (Fooks et al 2003), Africa (Swanepoel 2004) and Australia (Thompson 1999).

The virus is carried mainly by carnivores. In the final stages of the disease they excrete the virus in their saliva and transmit the disease to other animals when they bite them. Other forms of transmission such as aerosol transmission in bat colonies (Swanepoel 2004) and *per os* infection of kudu (Hubschle 1988) are rare exceptions. Following deposition in a bite wound the virus enters peripheral nerves and is transported through the nerves to the central nervous system. After entering the peripheral nerves the virus is not found in any other body tissues or in the blood. Amputation of limbs of mice experimentally infected in the foot pads has been shown to prevent the virus from progressing to the brain (Swanepoel 2004). The passage of virus through the nervous system is slow and, depending on the site of infection, the dose of virus and the animal concerned, the incubation period before the appearance of clinical signs may vary from weeks to years. The occurrence of viraemia is an exceptional event other than in experimental infections of young mice with large doses (Swanepoel 2004).

The virus spreads to the salivary glands at about the stage that there is generalised dissemination of infection in the brain. It then multiplies in the salivary glands and is excreted in the saliva. In the terminal stages, animals become uncoordinated and about 43% of infected horses become aggressive. The *Code* states that "For the purposes of the *Terrestrial Code*, the *incubation period* for rabies shall be 6 months, and the *infective*

period in domestic carnivores starts 15 days before the onset of the first clinical signs and ends when the animal dies". Typically, animals become ataxic and aggressive, or develop a paralytic form of the disease (Radostits et al 2007).

Rabies is a common disease of donkeys in Botswana (Segwagwe et al 1999). No reference was found about rabies in zebras.

11.1.5. Hazard identification conclusion

Rabies is a serious zoonotic, exotic, notifiable disease and is therefore classified as a potential hazard for the purposes of this risk analysis.

11.2. RISK ASSESSMENT

11.2.1. Entry assessment

Rare cases of transplacental infection may due to the immunosuppressive effects of pregnancy and have been demonstrated experimentally (Swanepoel 2004). However, viraemia and infection of organs other than the central nervous system do not occur except in the terminal stages when it is inconceivable that collection of embryos or semen would occur. Therefore, the likelihood of germplasm being infected with rabies virus is assessed to be negligible.

11.2.2. Risk estimation

The likelihood of release of virus in germplasm collected from clinically healthy horses is negligible. As a result, the risk estimate for rabies is negligible and it is not classified as a hazard in the commodities. Therefore, risk management measures are not justified.

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12. *Bacillus anthracis*

12.1. HAZARD IDENTIFICATION

12.1.1. Aetiological agent

Bacillus anthracis is a gram-positive spore-forming bacillus which causes the disease anthrax.

12.1.2. OIE list

Listed.

12.1.3. New Zealand status

B. anthracis is listed as an unwanted notifiable organism (MAF 2008).

12.1.4. Epidemiology

Anthrax occurs in a wide range of animals including horses, mules, donkeys and zebras (De Vos and Turnbull 2004). Horses, donkeys and zebras generally present with the acute form of anthrax that ends fatally within 72 hours of onset.

B. anthracis spores are released into the environment when carcasses of infected animals are opened and exposure to air causes the vegetative organism in the blood to sporulate. Spores can survive in the environment for many years. The disease occurs when spores are ingested by a susceptible animal (De Vos and Turnbull 2004).

The *Code* states, "There is no evidence that anthrax is transmitted by animals before the onset of clinical and pathological signs" and "For the purposes of the *Terrestrial Code*, the incubation period for anthrax shall be 20 days". The *Code* chapter on anthrax does not contain any recommendations relating to germplasm. In view of these facts it is inconceivable that anthrax could be transmitted in germplasm taken from healthy donors that have remained healthy for 28 days after collection of germplasm, as specified in the commodity definition.

12.1.5. Hazard identification conclusion

Since *B. anthracis* is not excreted in germplasm collected from healthy animals, it is not assessed to be a potential hazard in the commodity.

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13. *Leptospira* spp.

13.1. HAZARD IDENTIFICATION

13.1.1. Aetiological agent

The species *Leptospira interrogans* contains over 200 *Leptospira* serovars classified into 23 serogroups (Bolin 2008). A newer and alternative taxonomic scheme based on genomic characteristics classifies the pathogenic organisms into eight species, each of which is subdivided into serovars. For the purposes of this risk analysis the older method of classifying leptospires as serovars of *L interrogans* is used and serovars are written as if they were single species e.g. *L hardjo*, *L pomona* etc.

13.1.2. OIE list

Although Leptospirosis is listed by the OIE, from 2004 to 2009 the *Code* chapter did not contain recommendations for leptospirosis, only a statement that it was "under study". At the OIE General Session in May 2009, the International Committee accepted the recommendation of the Terrestrial Animal Health Standards Commission that the empty *Code* chapter on leptospirosis should be deleted from the *Code*.

13.1.3. New Zealand status

L hardjo, *L pomona*, *L balcanica*, *L copenhageni*, *L ballum* and *L tarrasovi* have been isolated from animals in New Zealand (Midwinter 1999). A single isolation of *L australis* has been reported from a human (Thompson 1980). In humans, serological diagnosis indicates that five of the species found in animals also infect humans, but *L balcanica,* which is associated with possums, has not been diagnosed in humans (ESR 2004). Other *Leptospira* spp. are classified by MAF as "other exotic organisms" (MAF 2008).

13.1.4. Epidemiology

Leptospira spp. occur in all countries assessed in this risk analysis but it is not possible to accurately define which serovars occur in each country.

Leptospirosis is not a single disease but a complex of diseases caused by many different leptospires. Most serovars are adapted to a particular host species with which they may co-exist for long periods without causing disease signs. Species other than the maintenance host may be more resistant to infection, but if infected are more susceptible to disease. In maintenance hosts, Leptospira localise in the kidneys and continue to be excreted in urine for protracted periods.

In horses, leptospiral infections may be associated with abortions, stillbirths, jaundice and inflammation of the eye (Hunter 2004).

In Canada and the USA the most common *Leptospira* in horses are *L Bratislava and L icterohaemorrhagiae.* In one investigation, 52% of horses over 7 years of age had antibodies to *L bratislava* (Kitson-Piggot and Prescott 1997) and, in another, 94% of horse had antibodies to *L icterohaemorrhagiae* (Lees and Gale 1994). The horse may be a

maintenance host for *L bratislava* (MAF 2000). Other serovars isolated from horses include *L pomona, L canicola, L hardjo* and *L grippotyphosa* (Ellis and O'Brien 1987). *L kennewicki* is often associated with abortions and still born foals (Poonacha et al 1993). Since *Leptospira* spp. can infect many species of mammals it is assumed that donkeys and zebras are also susceptible.

The organisms are shed in urine and infection can occur through mucous membranes or through the skin, particularly through abrasions and wounds, or can be transmitted orally or venereally (Hunter 2004). *Leptospira* spp. are excreted in semen and remain viable in semen prepared for artificial insemination (Hunter 2004; Masri et al 1997). Clinically diseased animals shed more organisms, and are more important sources of infection, than chronic carriers (Horsch 1989). Leptospira could not be cultured from embryos from infected heifers but leptospiral DNA associated with the embryos was demonstrated by PCR and leptospires were demonstrated by transmission electron microscopy. When the embryos were injected intravenously into heifers they did not stimulate antibody production (Bielanski et al 1998; Bielanski and Surujballi 1998). Apparently the organisms associated with the embryos were not viable.

The disease can be diagnosed by the isolation of the organism but, because this is a difficult process, it is more usually diagnosed by serological methods. A rising titre suggests a recent infection and a stable, often low-level titre indicates resolution or a chronic infection. The microscopic agglutination test is still the most commonly used herd test and a number of variations of ELISA are also available but generally lack serovar specificity (Bolin 2008). Leptospirosis is seldom the cause of economically serious disease in animals, but it is a zoonotic disease that occasionally causes serious disease in humans (Thornley et al 2002).

Leptospira spp. are sensitive to several antibiotics (Alt et al 2001; Gerritsen et al 1994; Gerritsen et al 1993; Hodges et al 1979; Murray and Hospenthal 2004; Oie et al 1983). Leptospiruria has been successfully treated with streptomycin (Alt et al 2001; Gerritsen et al 1994; Hodges et al 1979). Streptomycin and penicillin have been extensively used for prophylaxis and treatment of live animals, semen and embryos in international trade.

13.1.5. Hazard identification conclusion

Since *Leptospira* spp. that are exotic to New Zealand occur in the countries to which this risk analysis applies, they are assessed to be potential hazards in the commodities.

13.2. RISK ASSESSMENT

13.2.1. Entry assessment

Acutely infected animals or chronic carriers of infection may excrete the organism in their semen (Hunter 2004; Masri et al 1997). Therefore, the likelihood of entry in imported semen is non-negligible. Embryos are unlikely to transmit the infection but this has not been confirmed for horses. Therefore, the likelihood of transmission by embryos is assessed as non-negligible.

13.2.2. Exposure assessment

Imported embryos or semen would be transplanted or inseminated into naïve New Zealand mares. Therefore the likelihood of exposure is non-negligible.

13.2.3. Consequence assessment

If an exotic *Leptospira* serovar were to be introduced in imported equine germplasm, the direct consequences would be in the first instance confined to the recipient animals. The most likely clinical manifestations of this would be liver and kidney disease. Since horses are not known to become maintenance hosts, it is likely that any introduced new serovar would not persist in infected animals, and therefore would be unlikely to become established in the horse population. However, in the unlikely event that horizontal transmission to other horses were to occur, there may subsequently be sporadic cases of disease and reproductive failure in animals exposed to infectious urine.

For the same reasons, sporadic spread from horses to other animals and to humans could occur.

The establishment of a new *Leptospira* serovar to which humans are susceptible could lead to sporadic occurrence of leptospirosis in humans. About 100 cases of human leptospirosis occur each year in New Zealand. The majority of these cases are infected with *L hardjo* or *L pomona,* the serovars that occur most commonly in animals (ESR 2004). The number and seriousness of the cases would depend on the serovars involved and the possibility for contact with infected animals. Some serovars are not important as human pathogens e.g. in New Zealand *L balcanica* is common in its maintenance host the brush tailed possum, but infections of humans have not occurred despite close contact between possums and possum hunters (ESR 2004). It is concluded that the introduction of a new serovar in imported equine germplasm is unlikely to have a significant impact on the prevalence of cases of leptospirosis in domestic animals or humans.

There are not likely to be noticeable consequences for feral or wild animals but some species such as *L grippotyphosa*, *L canicola*, *L sejroe* and *L saxkoebing* could become established in mice and rats (Horsch 1989) and subsequently be responsible for infecting humans.

Therefore, the consequences of establishment of exotic letpospires are considered to be very low but non-negligible.

13.2.4. Risk estimation

Entry, exposure and consequence have all been assessed as non-negligible. As a result the risk estimate for exotic *Leptospira* serovars is non-negligible and they are classified as hazards in the commodity. Therefore, risk management measures can be justified.

13.3. RISK MANAGEMENT

13.3.1. Options

The following points should be considered when drafting options for the efficient management of exotic *Leptospira* serovars in the commodities:

- Because of the occurrence of long-term subclinical carriers of infection, quarantine of donors is not an effective measure.

- Diagnosis in donor horses by means of serology is complex to perform and the results are difficult to interpret because of the occurrence of cross reactions between serovars and the difficulty in interpretation of low titre reactions.
- Testing of semen samples by culture or PCR is problematic because isolation of organisms is difficult and the selection of primers for PCR that will recognise all serovars has not been described.
- *Leptospira* spp. 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* spp. by international trade. Treatment of embryos is also likely to be effective.
- The 2009 OIE General Session voted to delete the empty Code chapter on leptospirosis on the following grounds:

  > 'Leptospirosis is distributed globally; it is improbable that any country can, with any credibility, claim to be free from the disease. Further, it is unlikely that any country has an official control programme for leptospirosis. Current serological tests and culture techniques are not able, with any degree of confidence, to demonstrate that an animal is free from leptospirosis. Antibiotic treatment to clear renal carriage of leptospires is not consistently successful and has not been validated in all the species subject to international trade. Retention of this empty Chapter, with the words 'under study' gives the false impression that the OIE is able to formulate meaningful measures to manage the disease. '

Therefore the *Code* makes no recommendations for leptospirosis.

The available options, for both male and female germplasm donors, given in ascending order of stringency are:

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 MAF IHS for horse semen from countries covered by this risk analysis.

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14. *Taylorella* spp.

14.1. HAZARD IDENTIFICATION

14.1.1. Aetiological agent

Taylorella equigenitalis causes contagious equine metritis (CEM) in horses and other equidae. *Taylorella asinigenitalis* occurs in donkeys and in horses (Jackson and Heath 2006; Jang et al 2001).

14.1.2. OIE list

Listed.

14.1.3. New Zealand status

T. equigenitalis is listed as an unwanted notifiable organism (MAF 2008).

14.1.4. Epidemiology

Contagious equine metritis (CEM) has been reported from France, Germany, Finland, Sweden, Switzerland, the UK and recently from the USA in late 2008. The organism has been suspected but not confirmed in the Netherlands (OIE 2008). It has never been reported from Canada (OIE 2008), it occurred in Australia in 1978 (Hughes et al 1978) but is no longer present (OIE 2008). The organism is found in the UK but clinical manifestations of CEM have not been reported (OIE 2008) suggesting that non-virulent strains of the organism occur there. *T. asinigenitalis* has been reported from donkeys in the USA (Jang et al 2001) and from a stallion in Sweden (Baverud et al 2006). Its isolation was not associated with clinical signs of CEM.

CEM is usually transmitted venereally but also indirectly due to poor hygiene involving the use of contaminated water, utensils or instruments, contamination of the hands of staff or veterinarians, contact between the genital areas of mares, stallions or teasers, or nose to genital contact between stallions or teasers and mares. The disease can be transmitted in semen (Chanter 2004). In one report, embryos derived from semen from infected stallions were successfully implanted into recipient mares that did not subsequently become infected. The semen diluent contained penicillin and amikacin and the solutions used in the preparation of the embryos contained gentamicin (to which the particular strain was shown to be sensitive) and kanamycin (Hayna et al 2008). However, further work needs to be done before embryo transfer can be assessed to be a reliable method of preventing the transmission of *T. equigenitalis* by germplasm.

The disease is characterised by a mucopurulent vaginal discharge, 2-12 days after mating. There is a temporary endometritis and infertility. Recovered animals frequently become carriers (Chanter 2004; Heath and Timoney 2008).

There are no vaccines for the control of CEM and treatments with antibiotics cannot be relied upon to eliminate infections, particularly in mares (Chanter 2004).

The infection can be diagnosed by culturing swabs from suitable sites in the genital tract of mares and stallions. Sensitive PCR methods are available that can differentiate between *T. equigenitalis* and *T. asinigenitalis* (Anzai et al 2002; Blumink-Pluym et al 1994; Chanter 2004; Heath and Timoney 2008). The presence of *T. asinigenitalis* or non-virulent strains of *T. equigenitalis* is undesirable since these organisms could complicate the diagnosis of the disease. Serological tests are not useful for diagnosis (Heath and Timoney 2008).

No information is available about the occurrence of the organism in zebras, but it is likely that they could be infected if challenged.

14.1.5. Hazard identification conclusion

Since *T. equigenitalis* is a notifiable organism that occurs in several of the countries relevant to this risk analysis, it is assessed to be a potential hazard in the commodity. *T. asinigenitalis* should also be assessed to be a potential hazard.

14.2. RISK ASSESSMENT

14.2.1. Entry assessment

The organisms occur in several of the countries with which this risk analysis is concerned. Since they are excreted in semen and may be associated with embryos, the likelihood that they could be introduced in the commodities is non-negligible.

14.2.2. Exposure assessment

Imported embryos or semen would be transplanted or inseminated into naïve New Zealand mares. Therefore the likelihood of exposure is non-negligible.

14.2.3. Consequence assessment

Horses, donkeys and zebras are the only animals likely to be affected by the introduction of CEM. Wild horses could be similarly affected should they be exposed. There would be no consequences for humans as the virus is not zoonotic.

Since infection of stallions is asymptomatic, the consequences of introduction of CEM would be inflammation of the endometrium and the resulting temporary infertility that usually follows infection in mares. Since a proportion of recovered mares become carriers, infection could spread to stallions through mating such mares; infected stallions may shed the organism in semen for life. Therefore, the consequences for horse breeders would be non-negligible. Since this is a venereal disease, the likelihood of spread from infected horses to feral populations is remote.

Indirect consequences would include treatment of infected mares and if an official control programme were imposed there could be restrictions on animal movements. The major indirect consequence would be on international trade as several countries require certification of freedom from infection for horses that are exported from New Zealand. Horse exporters could be subjected to extra costs and inconvenience since additional measures may be imposed by trading partners.

Therefore the consequences are assessed to be non-negligible.

14.2.4. Risk estimation

Since entry, exposure and consequence assessments are all non-negligible, the risk is assessed to be non-negligible and *T. equigenitalis* and *T. asinigenitalis* are classified as hazards in the commodities. Therefore, risk management measures can be justified.

14.3. RISK MANAGEMENT

14.3.1. Options

When drafting options for the effective management of *T. equigenitalis* and *T. asinigenitalis* in the commodity the following relevant facts were assessed:

- *Taylorella* spp. are transmitted venereally.
- The organism infects equidae in some but not all of the countries of relevance to this risk analysis.
- No vaccines are available for prevention of the disease.
- Antibiotic treatment may be effective in some cases but cannot be relied upon to eliminate the organisms.
- Infection in horses can be diagnosed by culture or PCR. However, since culture is difficult it should be repeated at least three times at weekly intervals. Culture methods and collection and transport of swabs should follow the recommendations in the OIE *Manual of Diagnostic Tests and Vaccines* and should be carried out by laboratories specifically approved for the purpose (Heath and Timoney 2008).
- Both male and female donors should be free from infection.
- Embryo transfer shows promise as a method to prevent transmission of the organism from infected donors to recipients, but this requires further confirmation.

The *Code* does not make recommendations for equine genetic material. The *Code* recommendations for international trade of horses are:

Article 12.2.2.

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.

According to the *Code* an establishment should be considered to be infected until 2 months have elapsed since the confirmation of the last case and after the premises have been adequately cleansed and disinfected.

Options available for the effective management of *Taylorella* spp. in equine genetic material, in ascending order of stringency are:

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.

i) 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
ii) 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.

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15. *Burkholderia pseudomallei*

15.1. HAZARD IDENTIFICATION

15.1.1. Aetiological agent

Burkholderia pseudomallei (formerly *Pseudomonas pseudomallei*).

15.1.2. OIE list

Not listed.

15.1.3. New Zealand status

B. pseudomallei is listed as an unwanted exotic organism (MAF 2008).

15.1.4. Epidemiology

B. pseudomallei is the aetiogical agent of melioidosis, a disease of animals and humans. The disease is confined to tropical and subtropical regions, mainly in Asia and northern Australia and in some foci in Africa, the South Pacific and the Middle East (Groves and Harrington 1994; Inglis 2004; Inglis et al 2004). In the USA up to five cases occur each year in travellers and immigrants (CDC 2008). In the countries of relevance to this risk analysis it only occurs in tropical parts of Australia. A single imported case in a human has been reported in New Zealand (Corkill and Cornere 1987).

The organism is found in water and soil and is an opportunistic pathogen. It gains entry to animals through the oral mucosa, nasal mucosa or skin and has been transmitted by ingestion, parental inoculation and skin scarification (Groves and Harrington 1994). It is transmitted by direct contact with soil and water, especially through abrasions and wounds.(CDC 2008). Rare cases of person to person contact have been described including two cases of sexual transmission where chronic prostatitis occurred in the source patient (CDC 2008; Groves and Harrington 1994). However, prostatitis caused by *B. pseudomallei* and sexual transmission has not been reported in animals. Water was implicated as a possible source of infection in animals in six locations in one study (Inglis et al 2004).

In animals, clinical melioidosis is most commonly seen in sheep, goats and swine (Groves and Harrington 1994), but it also occurs in horses. In animals, the agent may cause a wide variety of signs varying from septicaemia and acute respiratory infections to localised abscesses. There is no evidence that semen or embryos transmit the infection in animals.

15.1.5. Hazard identification conclusion

B. pseudomallei is an organism that occurs in the environment and because of its known restricted distribution is unlikely to establish in New Zealand. It is an opportunistic pathogen and not known to be transmitted in germplasm. Therefore, it is not assessed to be a potential hazard in the commodities.

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16. *Salmonella* spp.

16.1. HAZARD IDENTIFICATION

16.1.1. Aetiological agent

There are approximately 2,500 known serovars in the *Salmonella* genus (Davies 2008). Most of these belong to the species *enterica* and the subspecies *enterica.* If correct naming conventions are used, serovar names such as Dublin and Typhimurium, which do not have species status, should not be italicised. The correct name for the serovar Typhimurium is *Salmonella enterica* subsp. *enterica* serovar Typhimurium. However, in this document *Salmonella* serovars are abbreviated to the format of *S* Typhimurium, *S* Enteritidis etc. and multiple serovars are referred to as *Salmonella* spp.

Within some serovars definitive phage types (DTs) can be identified. *S* Typhimurium DT104 is significant because it exhibits multiple resistance to the commonly used antibiotics and is a threat to human health (Hogue et al 1997; Jones et al 2002).

16.1.2. OIE list

Salmonella enteritidis and *Salmonella typhimurium* in poultry are listed under Section 6. which is titled "Veterinary Public Health".

The species specific serovars, *S* Gallinarum and *S* Pullorum, are included under diseases of poultry.

16.1.3. New Zealand status

S Abortusovis, *S* Dublin, *S* Gallinarum, and *S* Pullorum are listed as unwanted notifiable organisms. *S* Arizonae, *S* Enteritidis DT 4, *S* Typhimurium DT44 and DT104, and *Salmonella* spp. (exotic affecting animals) are listed as exotic unwanted organisms (MAF 2008).

Since 2003 *Salmonella* spp. isolated by medical and veterinary laboratories, from humans and animals, have been identified to serovar and phage type by the Environmental Science and Research (ESR) laboratory and recorded on a database (ESR 2008). In the year 2007 there were 1,341 isolates from humans comprising 140 serovar or phage types, and 1,001 isolates from non-human sources comprising 86 serovar or phage types.

S Typhimurium is frequently isolated from horses, other animals and humans. *S.* Typhimurium DT104 is isolated from humans only rarely and does not appear to be established in animals in this country (ESR 2008).

16.1.4. Epidemiology

Salmonellosis is an important infectious disease of horses and at least 40 different serovars have been isolated (Collett and Mogg 2004), the most commonly isolated being *S* Typhimurium. *S* Typhimurium DT104 was found to occur commonly in horses in the Netherlands (van Duijkeren et al 2002) and it occurs in Europe and North America (Davies

2001; Hogue et al 1997; Jones et al 2002). In Australia it occurs sporadically, often associated with imported cases (Helms et al 2005).

Salmonella spp. are generally transmitted by the oral route and factors such as infecting dose, the particular strain and serovar, and various stress factors influence the outcome of infection (Fenwick and Collett 2004). In horses, the infection is typically an enteric one which may present as peracute (mainly in young foals), acute or chronic forms. Young animals are more often affected than adults and very young foals may die after a short period of bacteraemia (Collett and Mogg 2004). Infected horses usually shed *Salmonella* spp. in their faeces for less than 30 days but shedding may continue for up to 300 days (Collett and Mogg 2004). Excreted organisms contaminate the environment and become a source of infection. Carriers of infections can be detected by culturing faecal samples but because excretion is intermittent, repeated sampling and culture are necessary (Collett and Mogg 2004).

The epidemiology of the host adapted strain *S* Abortusequi is quite different to the enteric Salmonellae. *S* Abortusequi causes abortions in pregnant mares and testicular lesions in stallions and fatal septicaemia in foals. It does not occur in the intestinal tract and has not been isolated from faeces (Collett and Mogg 2004). It appears to be spread between horses by contact with the products of abortion. Since infection may persist in the uterus of infected mares, venereal transmission is considered to be a possibility. While *S* Abortusequi has in the past been a common cause of abortion in horses, it is now extremely rare in the countries covered by this risk analysis. In the USA its prevalence declined to a point where isolations of the serovar had virtually ceased by 1970 (Collett and Mogg 2004). However, rare cases of *S* Abortusequi still occur in Europe (Madic et al 1997).

Although it is thought that *S* Abortisequi may be transmitted venereally, no information was found about infection of horse germplasm with any species of *Salmonella.* While it is theoretically possible that germplasm collected from septicaemic animals could contain *Salmonella* organisms, since such animals would be obviously ill, this is considered to be highly unlikely. The only other route by which germplasm could contain Salmonellae would be by contamination with faeces during collection.

IETS does not list *Salmonella* spp. in any risk category (IETS 2004).

16.1.5. Hazard identification conclusion

Many *Salmonella* spp. could infect horses and exotic strains may occur in exporting countries. Although there is no conclusive evidence to indicate that *Salmonella* spp. is transmitted by germplasm it is expected that *S* Abortisequi could be. In view of the above, exotic Salmonellae are considered to be potential hazards in the commodity.

16.2. RISK ASSESSMENT

16.2.1. Entry assessment

In view of its rarity in the countries considered in this risk analysis, the likelihood of *S* Abortisequi being present in donor animals is considered to be remote.

Infection of horses with enteric Salmonellae is relatively common, and most infections are asymptomatic. However, considering the standard methods of germplasm collection, the likelihood of faecal or environmental contamination is negligible. It is possible that germplasm collected from septicaemic donors could be infected.

Therefore the likelihood of entry of exotic *Salmonella* spp. is assessed to be very low but non-negligible.

16.2.2. Exposure assessment

Imported embryos or semen would be transplanted or inseminated into naïve New Zealand mares. Therefore the likelihood of exposure is non-negligible.

16.2.3. Consequence assessment

Direct consequences would in the first instance be confined to the recipient females that were inseminated or implanted with the imported genetic material. The most likely clinical effects of exotic salmonellae would be enteric disease in all age groups and septicaemia in foals. In the case of the horse-adapted strain *S* Abortisequi, there could be infertility in recipient mares. It is possible that infection may spread to other equine populations, including commercial breeders, horses used for recreation and small feral populations.

If an exotic *Salmonella* serovar were to become established in recipient animals then it would be likely to spread to other animal populations and to humans. A good example of how a *Salmonella* serovar spread in New Zealand has been demonstrated by the spread of *S* Brandenberg in sheep and humans (Clark et al 2004; Clarke and Tomlinson 2004). Production losses in animals and sporadic cases of salmonellosis in humans could occur. Wild and feral animals and birds may also be susceptible to infection. Another example was *S* Typhimurium DT160 which first manifested as outbreaks in sparrows with concurrent infections in humans (Alley et al 2002). Since neither of these widespread outbreaks resulted in reports of disease in native animals, it appears that the likelihood of spread to native animals is low.

The introduction of a multi-drug-resistant exotic serovar would be of considerable public health concern since treatment of human cases would become more difficult.

Indirect costs to horse owners would include treatment costs for sick animals, and inability to use, train or race animals while sick and recuperating from infection. In the case of a widespread severe outbreak, there could be some disruption to various equine events. In addition, there could be significant international trade consequences, particularly if the horse-adapted strain *S* Abortisequi were to become established, as a number of countries require certification of freedom from infection for horses that are exported from New Zealand. It is likely that some of these countries would impose further conditions on exports of horses from New Zealand.

Therefore the consequences are considered to be non-negligible.

16.2.4. Risk estimation

Since entry, exposure and consequence assessments are all non-negligible, the risk is assessed to be non-negligible and exotic *Salmonella* spp. are classified as hazards in the commodity. Therefore, risk management measures can be justified.

16.3. RISK MANAGEMENT

16.3.1. Options

The following points should be considered when drafting options for the efficient management of *Salmonella* spp. in the commodities:

- Considering the care that is normally taken in collection of germplasm, the likelihood of faecal contamination is very low.
- The horse-adapted serovar *S* Abortisequi is thought to be transmitted venereally, but its prevalence is now very low.
- Where reliable histories are available, selection of donors from properties on which salmonellosis has not occurred could increase the possibility of selecting donors that are not infected.
- For reliable diagnosis of carrier donors, culturing of faeces would need to be repeated three times at weekly intervals.
- The addition of antibiotics to germplasm may not be a completely reliable control measure because some strains of *Salmonella* may be resistant to certain antibiotics (Jones et al 2002; Wray et al 1991).
- Efficient methods of culturing *Salmonella* are available (Davies 2008). It is assumed that negative results, obtained when culturing processed germplasm that contains antibiotics, indicate that either *Salmonella* were not present or that the antibiotics have been effective in suppressing the growth of the organisms.

The *Code* does not contain measures to manage the risks posed by any salmonellae in horses or their genetic material.

Options for the effective management of *Salmonella* spp. in the commodity are:

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.

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17. *Histoplasma capsulatum* var. *farciminosum*

17.1. HAZARD IDENTIFICATION

17.1.1. Aetiological agent

Histoplasma capsulatum var. *farciminosum*.

17.1.2. OIE list

Listed.

17.1.3. New Zealand status

Unwanted exotic organism (MAF 2008).

17.1.4. Epidemiology

The disease known as epizootic lymphangitis or farcy occurs in horse's mules and donkeys. The disease has been reviewed (Picard and Vismer 2004) and information in this section was obtained from that source. The aetiological agent is the dimorphic fungus *Histoplasma capsulatum* var. *farciminosum*. The disease is characterised by nodular or ulcerative lesions in the skin and along lymphatics. It was a common disease when large numbers of horses were assembled in unhygienic conditions such as occurred in wars. The fungus is transmitted through skin lesions by contact with infected soil or pus. A conjunctival form of the disease is probably transmitted by flies and a respiratory form by inhalation. The organism can survive in soil for about 2 weeks. There is no evidence that the organism is transmitted by semen or embryos.

No evidence was found about farcy in zebras but they are likely to be susceptible if kept in unhygienic conditions.

17.1.5. Hazard identification conclusion

Since the organism is usually transmitted through skin or more rarely by other routes and transmission by semen or embryos has not been described, it is not assessed to be a potential hazard in the commodity.

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