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Manatū Ahu Matua

The risks associated with importing frozen pelleted semen

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Executive Summary

This document examines the risks associated with importing frozen pelleted semen from animal species that are currently subject to MPI import health standards (IHS) for semen. These IHSs exclude risk organisms from being present within the semen by imposing measures on the donors (e.g. testing to demonstrate freedom from infection) or the collected semen itself.

For this reason, this document is limited to an examination of the risk of contamination of pelleted semen during production and storage.

Specifically, frozen pelleted semen may be exposed to pathogens by the following means:

- During manufacture from the dry-ice block or associated equipment
- During cryopreservation from contaminated liquid nitrogen
- During storage from unhygienic flasks.

The risk management objective is to prevent semen pellets from being contaminated from external sources. Accordingly, the recommendations underpinned by this technical advice document can provide support for MPI's Imports Team when determining future import policy decisions in regards importing pelleted semen.

Background

Discrepancies have been identified across import health standards (IHSs) for cattle, horses, pigs, sheep and dogs regarding frozen pelleted semen.

For example, in the past these consignments have been refused clearance where the IHS refers to straws only. Moreover, pelleted semen is specifically excluded from IHSs for equine and porcine germplasm.

In contrast, pelleted semen imports are permitted by IHSs for canine and ovine germplasm.

A search of the FCS electronic filing system revealed no information that could justify the exclusion of pelleted semen from horse and pig IHSs. Additionally, Risk Analysis (Animals and Aquatic) are not aware of any assessments that have examined the risk of importing pelleted semen.

Nevertheless, several risk analyses have been developed for the importation of germplasm from various species. In general, the commodity definition states processing, packaging, storing, and transport of germplasm be in accordance with OIE *Code* recommendations.

Purpose

The Animal Imports Manager has requested a summary of the risks associated with the importation of frozen pelleted semen. The scope of this work is to include all animal species from all countries. Moreover, risk management options have been requested and a risk management recommendation made.

Therefore, the recommendation underpinned by this technical advice document can provide support for MPI when determining future import policy decisions in regards importing pelleted semen from anywhere in the world. This may lead to revised import requirements in IHSs.

Scope

This document examines the risks associated with importing frozen pelleted semen from any animal species. However, the IHSs for semen for each animal species exclude risk organisms from being associated with the commodity.

For this reason, this document is limited to an examination of the risk of contamination of pelleted semen during production and storage.

Risk of contamination

Frozen pelleted semen may be exposed to pathogens at the following stages of production and storage:

- During manufacture from the dry-ice block or associated equipment
- During cryopreservation in contaminated liquid nitrogen (LN_2)
- During storage from unhygienic flasks.

Contamination of the pellet during manufacture

For international trade, collected semen is generally stored frozen in polyvinyl straws that have been sealed or in a pelleted form within vials (preventing exposure to LN_2).

Frozen semen pellets are made by using a block of dry-ice (frozen carbon dioxide at -79 °C). The semen pellets are formed by making small depressions on the dry-ice block using a round heated end of a metal rod. Droplets of semen are dispensed into these indentations for freezing. After a few minutes, the frozen pellets are shaken loose from the dry-ice block and placed into a vial. Subsequently, the vial is plunged into LN_2 (-196 °C) to allow long-term storage.

A block of dry-ice cannot be hygienically cleaned. There is no barrier between the semen in the depressions and the dry-ice itself. Therefore, any residue of semen remaining in the indentations after the frozen pellet has been removed could theoretically pose a risk of cross-contamination. The literature reviewed has not explicitly examined this risk.

Contamination from liquid nitrogen

Examination of detritus from LN_2 that had stored germplasm from 6 to 35 years of continuous service recovered no viral agents, although a number of ubiquitous environmental bacterial organisms were isolated (Bielanski et al 2003). This study highlights a low potential for cross-contamination when specimens are improperly sealed. In another study, environmental bacteria and fungi were identified in samples of LN_2 used for storage of human embryos. However, Bielanski (2012) strongly emphasised that for cryopreserved human and animal embryos, no case of disease transmission has been reported which was attributable to storage in LN_2.

However, experimentally, it has been demonstrated that transfer of bacteria from infected semen pellets to sterile ones stored directly in LN_2 may occur. Piasecka-Serafin (1972) showed that sterile samples became infected with *Escherichia coli* and *Staphylococcus aureus* within 2 hours after placing in a container holding contaminated LN_2. More recently, Bielanski et al (2000) showed the potential for bovine viral diarrhoea virus and bovine herpes virus to be transmitted to unsealed stored embryos by experimentally contaminated LN_2. However, in this study none of the control embryos sealed in vials or straws were contaminated since they were not exposed to LN_2.

In general, air transportation of flasks that contain LN_2 are regulated as “hazardous material”. However, flasks that do not contain free LN but vapours only are referred to as “dry-

shippers". Dry-shippers are free from air transportation restrictions since they are classified as non-hazardous because there is no risk of LN_2 spilling during transportation. Experimentally, there was no transmission of selected bovine viral and bacterial pathogens from contaminated and non-contaminated embryos and semen stored in proximity in open containers in the vapour phase of LN within dry-shippers (Bielanski 2005b). Therefore, in contrast to flasks containing LN_2, dry-shippers are considered a safer means of transporting germplasm even in the proximity of pathogenic agents (Bielanski 2012). It has been suggested that it could be assumed that there is no or limited circulation of frozen particles within flasks by the LN_2 vapours in the absence of the liquid phase. The conventional LN_2 flasks allow extensive movement of both vapours and boiling LN_2 to occur (Bielanski 2012).

In conclusion, although experimental studies using contaminated LN_2 have infected unsealed semen pellets and embryos, there have been no compelling reports of disease transmission attributed to contaminated LN_2 documented by the International Embryo Transfer Society (IETS) for either semen or embryos (IETS 2010).

Moreover, a recent review of LN_2 as a source of contamination concluded that it can be assumed that the level of contamination of LN_2 is low and limited to ubiquitous organisms. Thus, the risk posed by contaminated LN_2 is remote (Bielanski 2012).

Contamination from storage in unhygienic flasks

The frequency of decontamination of LN_2 flasks is dependent on the flask volume, presence of infectious samples, number of stored samples, frequency of LN_2 refilling and amount of germplasm moved in and out. Non-sterile LN_2, common air pollutants and organisms attached to the outside of germplasm containers will contribute to the accumulation of contaminants in a flask over time. Such contamination usually involves non-pathogenic ubiquitous organisms (Bielanski 2005a).

A regulatory policy has not been established by the IETS for appropriate intervals between periodic decontamination of flasks.

Nevertheless, the IETS (2010) recommends periodic disinfection of flasks using an efficient disinfectant to diminish the likelihood of cross-contamination of samples during their storage. However, there are no published data on the efficacy of disinfectants for LN_2 flask decontamination. Manufacturers recommend sanitising flasks with solutions that do not react with aluminium or stainless-steel. In most cases, bleach, detergents, hydrogen peroxide solution or disinfectants such as DuPont™ Virkon® are suitable. Using a solution of 10% household chlorine bleach is often the best method for decontamination and is recommended by manufacturers.

For dry-shippers, decontamination is more difficult due to their inner construction (IETS 2010; Bielanski 2012). Nevertheless, the IETS (2010) presented the results of a study carried out by Bielanski (2005a) showing solutions of sodium hypochlorite or the application of gas sterilization using ethylene oxide as fully effective means of disinfecting dry-shippers.

OIE International trade recommendations

The OIE aims to provide transparency in the global animal disease and zoonosis situation and safeguards world trade by publishing health standards for international trade in the *Terrestrial Animal Health Code*.

Harmonisation ensures a consistent approach to addressing risks and means that countries should base their SPS measures on relevant international standards where they exist.

For international trade, it is recommended that semen pellets be placed into new LN_2 and separately from other genetic material that has not met the *Code* chapter requirements for collection and processing of bovine, small ruminant and porcine semen (OIE 2013).

Additionally, the OIE (2013) recommends storing semen pellets in a new or sterilised flask or container.

Risk management options

Dependent on the animal species concerned, the donor must have met the relevant semen IHS requirements. Therefore, the risk management objective is to prevent semen pellets from being contaminated from external sources.

To prevent cross-contamination from the dry-ice or any associated equipment during pellet manufacture, one or a combination of the following measures could be considered in order to achieve the risk management objective:

1. Certification that pelleted semen has been prepared in a manner to prevent cross-contamination whereby no contact with any other semen has occurred.

   N.B. For each donor, a dry-ice block must be for single use only, and any associated equipment (pipettes etc.) disinfected or single use only.

2. A dry-ice block may be used for multiple donors so long as donors are of an equivalent health status.

Storing infected pellets with sterile pellets may cause cross-contamination if they are not properly sealed or as a result of accidental breakage. Infected pellets that expose and contaminate LN_2 may enable the transfer of pathogens to other pellets. One or a combination of the following measures could be considered in order to achieve the risk management objective:

1. Vials should be stored into containers or flasks with new (fresh) LN_2.

2. Vials are sealed to prevent pellets from exposure to LN_2.

3. Containers or flasks contain semen pellets only from donors of an equivalent health status.

To prevent contamination of pellets from storage in unhygienic flasks, one or a combination of the following measures could be considered in order to achieve the risk management objective:

1. Semen pellets should be stored in sanitised flasks or containers.

2. Semen pellets should be stored in sterilised or new flasks or containers.

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