Biosecurity New Zealand
Tiakitanga Pūtaiao Aotearoa

Import Risk Analysis

Bovine leukaemia virus and *Campylobacter fetus* subspecies *venerealis* in bovine frozen semen, *in-vivo* derived and *in-vitro* produced embryos

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Import risk analysis: Bovine leukaemia virus and *Campylobacter fetus* subspecies *venerealis* in bovine frozen semen, *in-vivo* derived and *in-vitro* produced embryos

Version 1.0

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Approved for IHS development

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New Zealand is a member of the World Trade Organisation and a signatory to the Agreement on the Application of Sanitary and Phytosanitary Measures ("The Agreement"). Under the Agreement, countries must base their measures on an International Standard or an assessment of the biological risks to plant, animal or human health.

This document provides a scientific analysis of the risks of bovine leukaemia virus and *Campylobacter fetus* subspecies *venerealis* in bovine frozen semen, *in-vivo* derived and *in-vitro* produced embryos. It assesses the likelihood of entry, exposure, establishment and spread of these agents in relation to imported bovine frozen semen, *in-vivo* derived and *in-vitro* produced embryos and assesses the potential impacts of those organisms should they enter and establish in New Zealand. The document has been internally and externally peer reviewed.

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Acronyms and abbreviations

Term/Abbreviation	Definition
AGID	Agar gel immunodiffusion
AI	Artificial Insemination
BGC	Bovine genital campylobacteriosis
BLV	Bovine leukaemia virus
Cff	*Campylobacter fetus* subspecies *fetus*
Cfv	*Campylobacter fetus* subspecies *venerealis*
Code	Terrestrial Animal Health Code of the World Organisation for Animal Health
DAWR	Department of Agriculture and WaterResources
EBL	Enzootic bovine leukosis
ELISA	Enzyme linked immunosorbent assay
ICTV	International Committee on Taxonomy of Viruses
IETS	International Embryo Technology Society
IFAT	Immunofluorescence antibody test
IVD	*In-viv*o derived
IVP	*In-vitro* produced
MAF	Ministry of Agriculture and Forestry
MLST	Multilocus sequence typing
MPI	Ministry for Primary Industries
OIE	The World Organisation for Animal Health
PCR	Polymerase chain reaction
PL	Persistent lymphocytosis
SPS	Sanitary and phytosanitary
USDA	United States Department of Agriculture
VMAT	Vaginal mucus agglutination tests

1. Executive summary

This document is a qualitative analysis of the risk posed by bovine leukaemia virus (BLV), the causative agent of Enzootic bovine leukosis (EBL) and *Campylobacter fetus* subspecies *venerealis (Cfv),* the causative agent of bovine genital campylobacteriosis (BGC), in imported frozen bovine semen and *in-vivo* derived and *in-vitro* produced embryos.

The methodology for this risk analysis follows the guidelines as described in *Biosecurity New Zealand Risk Analysis Procedures – Version 1* and in Chapter 2 of the *Terrestrial Animal Health Code* of the World Organisation for Animal Health (hereafter referred to as the *Code*).

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

Accordingly, risk management options have been provided which include one or a combination of the following measures:

1. the exporting country is free of EBL
2. the collection, processing, storage and transport of semen in accordance with the *Code;*
3. testing of processed semen by an approved virus isolation or polymerase chain reaction test;
4. the donor bull was resident at the time of semen collection in an EBL-free herd and that the bull has been tested for BLV before and after collection, on blood samples with negative results.

The likelihood of BLV being present in *in-vivo* derived or *in-vitro* produced embryos is assessed to be negligible and therefore the risk estimate is negligible. BLV is not assessed to be a risk in *in-vivo* derived (IVD) or *in-vitro* produced (IVP) embryos.

The likelihood of *Cfv* being present in semen is assessed to be high. The likelihood of subsequent exposure and transmission of *Cfv* to susceptible animals is assessed to be high. The consequences of entry and establishment of *Cfv* are assessed to be non-negligible. *Campylobacter fetus* subspecies *venerealis* is therefore assessed to be a risk in imported bovine semen.

Accordingly, risk management options have been provided which include one or a combination of the following measures:

1. a minimum 28-day isolation period for donor bulls prior to entry to a semen collection facility;
2. testing of preputial samples of donor bulls during the pre-entry isolation period;
3. bulls and teasers resident at semen collection facilities are to have a preputial sample tested for *Cfv* at least annually with a negative result shown.

The likelihood of *Cfv* being present in *in-vivo* derived or *in-vitro* produced embryos is assessed to be very low. The likelihood of exposure and subsequent transmission of *Cfv* to susceptible animals via embryos is assessed to be negligible and therefore the risk estimate is negligible. *Cfv* is not assessed to be a risk in *in-vivo* derived or *in-vitro* produced embryos.

2. Introduction

An import risk analysis was completed in 2009 to assess the risk due to disease-causing organisms associated with the importation of bovine semen and embryos. This risk assessment classified BLV and *Cfv* as endemic, non-notifiable organisms in New Zealand. Consequently these agents were not assessed to be hazards (MAF, 2009).

There have been no detections of BLV in New Zealand since the last BLV-infected dairy cows were culled in 2007 (Voges, 2012).

The disease bovine genital campylobacteriosis (BGC) is caused by the organism *Cfv*. The last reported diagnosis of *Cfv* by culture in New Zealand was in 1992. Diagnoses of *Cfv* since this case have been based on three tests (VMAT, ELISA, and McMillen-PCR) that have subsequently been found to be unsatisfactory under New Zealand conditions (McFadden and Heuer, 2011; McFadden, 2010).

The Ministry for Primary Industries (MPI) passive surveillance system which monitors the disease diagnoses by the veterinary health laboratories in production animals in New Zealand has had no confirmed reports of BLV since 2008 nor *Cfv* since 1992.

Both BLV and *Cfv* were added to the Notifiable List in 2016. New Zealand currently reports to the OIE that EBL and BGC have been absent since 2008 and 1993 respectively from both the national dairy and beef herds.

This risk analysis has been developed in response to a request from the Animals Import Team to review the risk of BLV and *Cfv* associated with the importation of bovine semen and *in-vivo* derived (IVD) and *in-vitro* produced (IVP) and the available measures that could effectively manage this risk.

3. Scope and commodity definition

This risk analysis is limited to the description of the risks due to BLV and *Cfv* associated with the importation of frozen semen and IVD and IVP, embryos from cattle (*Bos indicus* and *B. taurus),* from all countries.

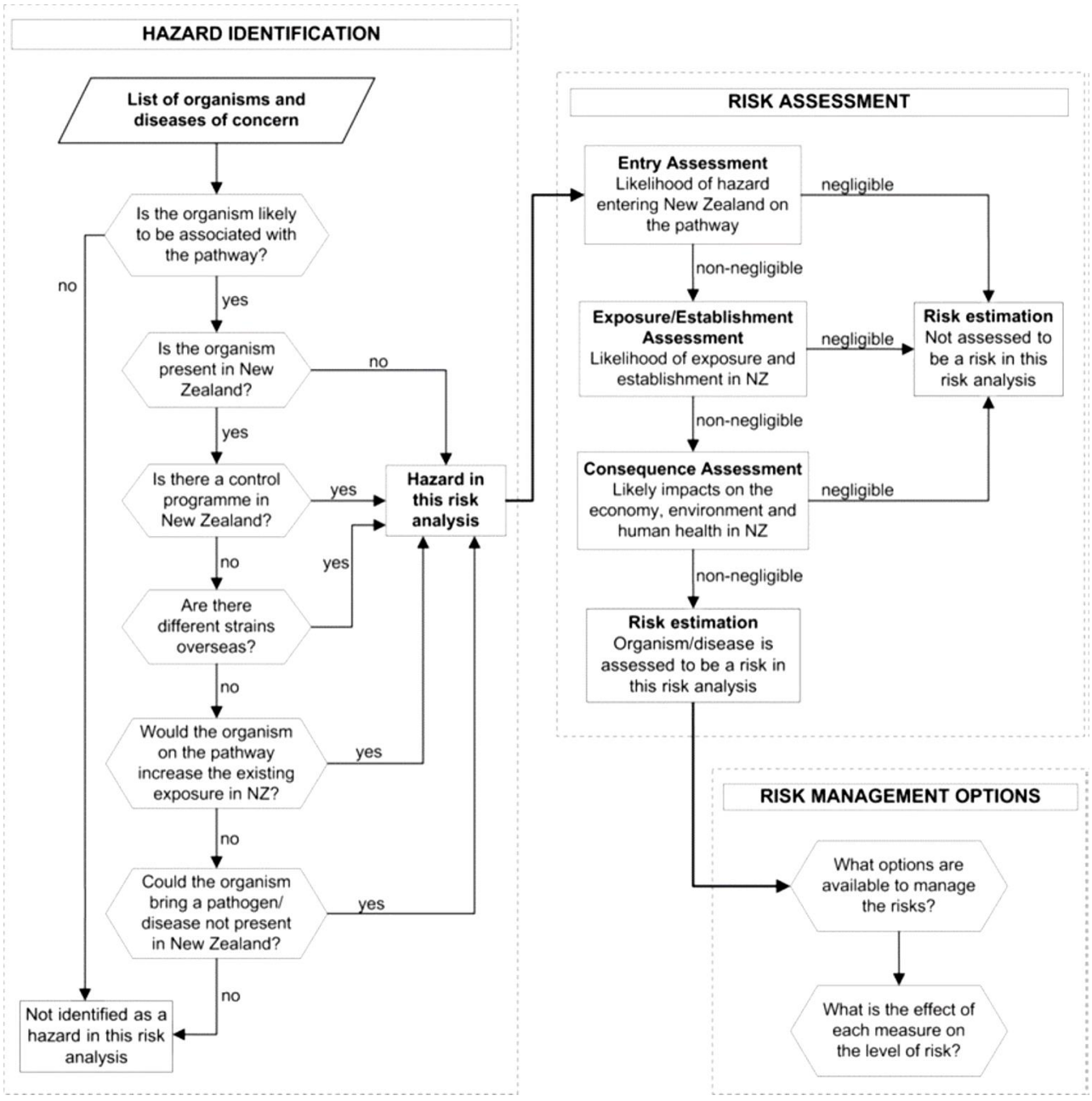
The commodity definition for this risk analysis includes that;

- The oocytes used for producing IVP embryos are to be collected from live donor cows or heifers with a known health status, and not from abattoir collected ovaries.
- All semen, oocytes, IVD embryos and IVP embryos are to be collected, processed, stored and transported in accordance with the relevant parts of Chapters 4.5, 4.6, 4.7 and 4.8 of the *Code*.
- Bovine germplasm includes semen, embryos (both IVD and IVP), oocytes and somatic cells. This document does not include assessing risks associated with importing oocytes and somatic cells, nor the risks associated with micromanipulated oocytes or embryos i.e. oocytes or embryos which have been subjected to biopsy, splitting, transgene injection, intracytoplasmic sperm injection, nuclear transfer or other interventions which breach the integrity of the zona pellucida.

4. Risk analysis methodology

The methodology used in this risk analysis follows the guidelines as described in *Biosecurity New Zealand Risk Analysis Procedures – Version 1* and in Chapter 2 of the *Code*. The process followed is shown in Figure 1.

Figure 1. The risk analysis process



4.1 Hazard Identification

Hazard identification includes formal identification of the organism, whether it is the cause of an OIE listed disease, its New Zealand status, and a discussion on the relevant aspects of the epidemiology and characteristics of the organism. The hazard identification section is concluded by a determination of whether or not the organism is identified as a hazard or not. All agents identified as hazards are subjected to risk assessment.

4.2 Risk Assessment

Risk assessment consists of:

a) *Entry assessment*: The likelihood of a hazard (pathogenic organism) being imported with the commodity.

b) *Exposure assessment*: Describes the biological pathway(s) necessary for exposure of susceptible animals or humans in New Zealand to the hazard.

c) *Consequence assessment*: Describes the likely potential consequences of entry, exposure and establishment or spread of an imported hazard.

d) *Risk estimation*: An estimation of the risk posed by the hazard associated with importing products. This is based on the entry, exposure and consequence assessments. If the risk estimate is assessed to be non-negligible, then the hazard is assessed to be a risk and risk management measures may be justified to reduce the level of risk to an acceptable level.

Not all of the above steps may be necessary in all risk assessments. The OIE methodology makes it clear that if the likelihood of entry is negligible for a certain 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 when the likelihood of entry is non-negligible but the exposure assessment concludes that the likelihood of susceptible species being exposed is negligible, or when both entry and exposure are non-negligible but the consequences of introduction are assessed to be negligible.

4.3 Risk Management

For each organism assessed to be a risk, options are identified for managing that risk. Recommendations for the appropriate sanitary measures to achieve the effective management of risks are not made in this document. These will be determined when the IHS and risk management proposal documents are drafted.

As obliged under Article 3.1 of the World Trade Organization's 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. That is, 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.

5. Bovine leukaemia virus

5.1 HAZARD IDENTIFICATION

5.1.1 Aetiological agent

Family: *Retroviridae* Genus: *Deltaretrovirus* Species: Bovine leukaemia virus (ICTV 2016)

5.1.2 OIE list

Enzootic bovine leukosis (EBL) is an OIE listed disease (OIE 2018a). Bovine leukaemia virus (BLV) is the causative agent of EBL.

5.1.3 New Zealand status

Serological surveys carried out in New Zealand in 1979, 1988, 1989 and 1990 identified 0.05% (3 of 6,000), 0.27% (69 of 25,780), 0.28% (40 of 14,188), and 0.09% (10 of 11,357) seropositive animals respectively. The samples from 1988, 1989 and 1990 consisted of submissions for export and admission to AI breeding centres. It is likely that the low seroprevalence of 0.09% in 1990 can be attributed to the fact that exporters had begun to select their export cattle from herds known to be uninfected (Hillbink and Penrose, 1993).

In 1991 a random sample of 4,468 bulk milk samples were tested using an ELISA and identified 105 (2.4%) herds as positive (Hillbink and Penrose, 1993).

The New Zealand dairy industry implemented the Dairy Enzootic Bovine Leukosis Control Scheme in 1997. The last BLV-infected dairy cows were culled in 2007–2008 and annual screening of the dairy industry was maintained at over 50% of all herds for several years (Voges, 2012).

Bovine leukaemia virus is notifiable under the Biosecurity (Notifiable Organisms) Order 2016. There has been no laboratory confirmed case of EBL reported since 2008 in dairy or beef herds (OIE 2018b). BLV is subject to passive surveillance activities.

5.1.4 Epidemiology

Distribution of disease

Enzootic bovine leukosis has a wide global distribution and is present in cattle populations across the world (OIE, 2018b). Some countries such as those in Western Europe have successfully eradicated EBL, whilst others have implemented programmes to achieve this (European Commission, 2014). In Australia, EBL was successfully eradicated from the dairy cattle herd in 2013 and is presently at a very low prevalence in the national beef herd (Animal Health Australia, 2016). In contrast, sero-epidemiological surveys in countries such as the US, Canada, Argentina, Japan, and Korea demonstrate herd prevalence as high as 84%, 89%, 84%, 68% and 86% respectively (USDA, 2008; Van Leeuwen et al, 2001; Van Leeuwen et al, 2005; Van Leeuwen et al, 2006; Trono et al, 2001; Murakami et al, 2001; Suh et al, 2005).

Pathogenesis

Natural infection with BLV occurs in cattle (*Bos taurus* and *Bos indicus*). Sheep are susceptible to experimental infection and the development of lymphoma (OIE, 2017a). Infection of natural hosts with BLV induces a persistent lifelong infection.

Infection with the virus in cattle is lifelong and gives rise to a persistent antibody response. Antibodies can first be detected 3-16 weeks after infection (OIE, 2017a). The severity of the disease syndrome EBL, resulting from infection with BLV, can range from subclinical to clinical. The majority of infected animals, approximately 70%, become subclinical carriers of the virus (Schwartz and Levy, 1994). Approximately 20-30% of infected animals develop persistent lymphocytosis (PL), a benign condition characterised by a proliferation of B lymphocytes. Cases of PL do not develop clinical signs although haematological alterations are present. A small number, less than 1-5%, of animals develop the clinical form of EBL characterised by the development of lymphoid tumours (Schwartz and Levy, 1994). This is seen predominantly in cattle older than 4-5 years of age.

Although infection with BLV does not cause significant pathology unless infection progresses to the tumour development stage, there is evidence to support the hypothesis that BLV infection at any stage (subclinical carrier, persistent lymphocytosis or tumour development) can cause abnormal function of the immune system (Frie and Coussens, 2015).

Transmission

Bovine leukaemia virus is transmitted through the transfer of virus infected lymphocytes. One of the most significant transmission pathways is iatrogenic spread through the use of contaminated needles, surgical equipment, rectal gloves and, artificial insemination (AI) equipment (Wrathall et al, 2006). Other routes of infection include transplacental infection during pregnancy (Agresti et al, 1993; Lassauzet et al, 1991) and mechanical transmission by blood-sucking insects such as tabanids (Perino et al, 1990; Ohshima et al, 1981). The feeding of colostrum/milk to calves has been reported as a potential route of transmission. However, given that some studies have demonstrated the protective effect of colostrum fed to calves born to BLV positive dams, it is apparent that our understanding of the dynamics of the infectious versus the protective quality of colostrum from BLV-positive cows remains incomplete (Gutierrez et al, 2015; Nagy et al, 2007).

Diagnosis

Infected animals can be identified through detection of the agent or detection of an immune response. Methods for detection of BLV include virus isolation (VI) or antigen detection by polymerase chain reaction (PCR) or electron microscopy, whilst methods of antibody detection include agar gel immunodiffusion (AGID) of serum samples and enzyme-linked immunosorbent assay (ELISA) of serum or milk samples. Both the AGID and the ELISA are prescribed tests for international trade (OIE, 2017a).

Vaccination

There is currently no vaccine available commercially for the control of EBL (OIE, 2017a). Previous attempts to obtain a vaccine against BLV were unsuccessful in herd conditions, mainly because of incomplete or transient stimulation of the host immune response (Gutierrez et al, 2014).

Bovine leukaemia virus in germplasm

Although BLV has been detected by PCR in the semen of seropositive bulls (Jafari and Asadpour, 2010; Dus Santos et al, 2007) this appears to occur infrequently (Choi et al, 2002). The presence of the virus in semen occurs through the leakage of BLV infected lymphocytes into the genital tract (Afshar and Eaglesome, 1990) and therefore requires some level of compromise to the reproductive tract.

Experimental studies have demonstrated transmission of BLV following *in-utero* inoculation of infected lymphocytes (Van Der Maaten and Miller, 1978) and infected lymphocytes mixed with semen (Roberts et al, 1982). These experimental studies support the hypothesis that semen, contaminated with infected lymphocytes, may act as a vehicle for virus transmission.

However, there are several studies, both field and experimental, which demonstrate that the likelihood of transmission of BLV via semen is actually very low.

Monke (1986) reported the maintenance of a BLV-free status for 5 years in a BLV-seronegative closed-herd despite semen from seropositive bulls being used for AI.

In a study by Belev and others (1986), semen from seropositive bulls was used to breed BLV seronegative cows and heifers by AI. The authors reported that serologic investigations of both dams and calves revealed no BLV antibodies and concluded that transmission of BLV via contaminated semen during artificial insemination was an unlikely pathway.

Miller and Van Der Maaten (1979) collected semen from bulls with persistent BLV infection, as identified by serological examination and the demonstration of BLV in peripheral lymphocytes, and inoculated weanling sheep. The authors reported that the inoculated sheep did not develop BLV antibodies.

Despite the evidence demonstrating the low likelihood of transmission via semen, the *Code* provides recommendations for the importation of bovine semen. The *Code* requires that an exporting country certifies that the donor bull was resident at the time of semen collection in an EBL-free herd and that the bull has been tested for BLV before and after collection, on blood samples with negative results.

There are several studies which have investigated the potential of *in-vivo* derived and *in-vitro* produced embryos to retain and transmit BLV.

Bouillant and others (1982), examined embryos from BLV-positive donor cows for the presence of the virus and reported no detections of the agent. Di Giacomo and others (1986) investigated transmission of BLV by embryo transfer and found that one hundred and sixteen embryos transplanted from BLV-positive donor cows into BLV-negative heifers produced serologically negative progeny. In addition, the recipient dams all remained BLV-seronegative. In a similar study, Kaja and others (1984) reported that BLV-seronegative cows receiving embryos from BLV-seropositive donors were seronegative at 21 months for BLV antibodies. Furthermore, the 30 resultant calves born from the successful transfers also remained seronegative. In a study by Eaglesome and others (1982), 151 embryos were collected from BLV infected donors and transferred to uninfected recipients. All of the recipients, pregnant and non-pregnant, and all of the 26 live calves produced remained serologically negative for antibodies to BLV-glycoprotein antigen.

Enzootic bovine leukosis is assessed by the International Embryo Technology Society (IETS) and is described by the *Code*, Article 4.7.14., as a Category 1 disease i.e. those for which sufficient

evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer in accordance with the IETS Manual.

Less research has been performed in investigating the potential for *in-vitro* produced embryos to be contaminated with BLV and to retain and transmit infection. Bouillant and others (1982), examined unfertilized ova from BLV-positive donor cows for the presence of the virus and reported no detections of the agent. Bielanski and others (2000) investigated the potential for *in-vitro* produced embryos to retain BLV infectivity. The authors reported that washing was effective in removing BLV from 170 matured oocytes which were artificially exposed to BLV contaminated semen. In a similar study by Hare and others (1985), nine zona-intact ova were exposed *in-vitro* to BLV, washed and then tested for the presence of the virus. The authors reported that BLV was not detected in association with the washed ova.

5.1.5 Hazard identification conclusion

Bovine leukaemia virus is the causative agent of EBL, an OIE listed disease. The agent has been isolated in germplasm. New Zealand has eradicated the disease from the national dairy herd through the implementation of the Dairy Enzootic Bovine Leukosis Control Scheme. The organism is notifiable in New Zealand.

Bovine leukaemia virus is identified as a hazard in the commodity.

5.2 RISK ASSESSMENT

5.2.1 Entry assessment

Semen

Infection with BLV induces a lifelong carrier state. Infected animals remain subclinical in the majority (70%) of cases. Semen can be contaminated with BLV when virus infected lymphocytes leak into the genital tract. BLV DNA has been detected by PCR in the semen of infected bulls.

The likelihood of entry is assessed to be low.

In-vivo derived embryos

Bovine leukaemia virus has not been isolated from IVD embryos collected from sero-positive donors (Bouillant et al, 1982). Experimental studies have demonstrated that washing protocols are sufficient to remove embryo associated BLV (Hare et al, 1985).

Enzootic bovine lukosis has been assessed by the IETS and is described as a Category 1 disease i.e. those for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled between collection and transfer in accordance with the IETS Manual.

The likelihood of entry is therefore assessed to be negligible for IVD embryos prepared in accordance with the IETS Manual.

In-vitro produced embryos

Bovine leukaemia virus has not been isolated from ova collected from sero-positive donors (Bouillant et al, 1982). Experimental studies have demonstrated that washing protocols are sufficient to remove oocyte associated BLV (Bielanski et al 2000; Hare et al 1985).

Since washing as per the IETS recommendations has been demonstrated to remove the virus, the likelihood of entry is assessed to be negligible.

5.2.2 Exposure assessment

Semen

The likelihood of exposure is certain since imported semen is inseminated into susceptible females. Venereal transmission of BLV via bovine semen, by natural service and artificial insemination, has not been demonstrated. However, the potential for infected lymphocytes, which may be present in semen, to cause infection following experimental *in-utero* inoculation has been demonstrated. The likelihood of transmission should BLV be present in semen has thus been shown to be possible (Roberts et al, 1982).

Given that infection in 70% of animals is subclinical and not associated with clinical signs or haematological alterations it is wholly plausible that semen collected from an apparently clinically healthy bull could harbour the organism.

Furthermore, despite the evidence demonstrating the low likelihood of transmission via semen, the OIE considers the risk of BLV in bovine semen significant enough to warrant risk mitigation and consequently provides *Code* recommendations for the importation of this commodity. The current standard practice for internationally traded semen is to blood test donor bulls for infection with BLV before and after collection to show they are not infected.

The likelihood of exposure is therefore assessed to be low.

5.2.3 Consequence assessment

Herds infected with BLV experience economic losses due to clinical lymphosarcoma and the associated costs of loss of genetic potential and increased costs of replacements due to culling. In addition, infection with BLV decreases animal productivity through decreased cow longevity (Nekouei et al, 2016; Bartlett et al, 2013; Erskine et al, 2012), decreased milk production (Norby et al, 2016; Yang et al, 2016) and impaired immune function (Frie and Coussens, 2015; Sandev et al, 2004). Significant economic losses occur as a consequence of this compromised animal productivity (Ott et al, 2003).

Enzootic bovine leukosis is an OIE listed disease which has been eradicated from a significant number of New Zealand's trading partners. Trade restrictions on live animals and germplasm are likely to apply to animals and animal products sourced from BLV infected herds.

Studies investigating the zoonotic potential of BLV were performed in the 1970s and 1980s. These studies examined serum samples from 1,761 humans, including cancer patients, farm workers, and veterinarians for the presence of BLV antibodies. Having detected no antibodies against BLV in

human serum samples, the author concluded that there was no epidemiological or serological evidence from human studies to indicate that BLV is a zoonotic agent (Burridge, 1981).

More recent studies report detection of BLV antibodies in 74% of 257 human serum samples (Buehring et al, 2003). The significance of these findings remains unclear given that the study could not determine whether the antibodies were a response to infection or merely to heat-inactivated BLV consumed in food products (Buehring et al, 2014). Reports from other studies that sera from humans infected with human T-lymphotropic virus type 1 and type 2, retroviruses closely related to BLV, cross-react with BLVp24 due to an epitope shared by the related viruses adds further uncertainty to the significance of these findings (Onuma et al, 1987).

Further evidence for the possible transmission of BLV from cattle to humans was the detection of BLV sequences by PCR in 44% of human breast tissue (Buehring et al, 2014).

Despite these findings there remains a lack of conclusive evidence for BLV as a human pathogen. The impacts to human health are therefore assessed to be negligible.

Natural infection with BLV occurs only in cattle (*Bos taurus* and *Bos indicus*), water buffalo and capybara. The vast majority of cases are subclinically infected and consequently the virus is associated with minimal animal distress or significant pathology unless infection progresses to the tumour development stage. The impacts of BLV to animal health in New Zealand are limited to cattle and water buffalo and are assessed to be low.

There are no capybara present in New Zealand, and no wild animals here are susceptible to infection. Accordingly, the impact to wildlife and the environment is assessed to be negligible.

5.2.4 Risk estimation

Since the entry, exposure and consequence assessments for BLV in semen are non-negligible, BLV is assessed to be a risk in imported semen. Consequently, risk management measures can be scientifically justified.

Since the likelihood of entry of BLV in IVD and IVP embryos is assessed to be negligible, the risk estimate is also assessed to be negligible. Accordingly BLV is not a risk in IVD and IVP embryos. Risk management measures are therefore not required.

5.3 RISK MANAGEMENT

Embryos that meet the commodity definition (processed in accordance with IETS recommendations), require no additional risk management since the risk estimate is negligible.

This section will not discuss embryos further, but will focus on managing the risk of introducing BLV associated with semen.

Accordingly, the following points were taken into account when describing options for effectively managing the risk in semen.

- Enzootic bovine leukosis is an OIE listed disease (OIE 2018a).
- Bovine leukaemia virus, the causative agent of EBL is notifiable under the Biosecurity (Notifiable Organisms) Order 2016.

- Bovine leukaemia virus is subject to passive surveillance in New Zealand. There has been no laboratory confirmed isolation of the agent since 2007.
- Bovine leukaemia virus DNA has been isolated in the semen of BLV-seropositive bulls.
- Transmission of BLV occurs through the transfer of infected lymphocytes.
- Infection in approximately 70% of animals is subclinical and not associated with clinical signs or haematological alterations.
- Methods of diagnosis prescribed by the OIE for international trade in donor animals include the AGID of serum samples.
- The *Code* Chapter 11.6 includes recommendations for the importation of bovine semen and oocytes or embryos (OIE 2017b).

Options

Option 1

At the time of semen collection for export to New Zealand, the exporting country was free of EBL. A country may be considered free from EBL provided it meets the general principles in Chapter 1.4. and that no cases of EBL or detections of BLV have occurred in the past two years.

Option 2

The semen was collected, processed, stored and transported in accordance with Chapters **4.5.** and **4.6.** of the *Code.*

N.B. This is the current import health measure.

Option 3

The semen was collected, processed and stored in accordance with Chapters **4.5.** and **4.6.** of the *Code*; and

An aliquot of not less than 0.5 ml of processed semen from the final collection of each donor was tested by an approved VI test or a PCR test with negative results.

N.B. This option would likely further reduce the probability of infected semen over and above that achieved by Option 1 alone. The testing of semen is not as reliable as testing the donor bull.

Option 4

- The semen was collected, processed and stored in accordance with Chapters **4.5.** and **4.6.** of the *Code.*
- The donor bull was resident at the time of semen collection in an EBL free herd in accordance with the *Code*; and

- if less than two years of age, the bull came from a serologically negative 'uterine' dam; or
- the bull was subjected to diagnostic tests for BLV on blood samples on two occasions with negative results, the first test being carried out at least 30 days before and the second test at least 90 days after collection of the semen. (Both the AGID and the ELISA are prescribed tests for international trade (OIE, 2017a).

N.B. This option aligns with the *Code* recommendation for the importation of bovine semen.

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6. Campylobacter fetus subspecies *venerealis*

6.1 HAZARD IDENTIFICATION

6.1.1 Aetiological agent

Campylobacter fetus is a gram negative bacterium which includes the subspecies *Campylobacter fetus* subspecies *venerealis (Cfv)* and *Campylobacter fetus* subspecies *fetus (Cff)* (Michi et al, 2016). The subspecies *Cfv* exclusively inhabits the genital tract of cattle and is the recognised cause of bovine genital campylobacteriosis (BGC), a disease characterised by temporary infertility and abortion (Michi et al, 2016). The subspecies *Cff* generally inhabits the intestine but may also be isolated from the genital tract and is associated with abortion in sheep, and occasionally cattle.

6.1.2 OIE list

Bovine genital campylobacteriosis is an OIE listed disease (OIE 2018). The causative agent of BGC is *Campylobacter fetus* subsp. *venerealis (Cfv).*

6.1.3 New Zealand status

Campylobacter fetus subsp. *venerealis* is notifiable under the Biosecurity (Notifiable Organisms) Order 2016 and is subject to passive surveillance activities. Although the agent has previously been detected in New Zealand (Loveridge and Gardner, 1993), there has been no confirmed case (by culture) in the national dairy or beef herds reported since 1992 (OIE WAHIS 2018).

Detections of *Cfv* reported in New Zealand after the detection by Loveridge and Gardner in 1993 have been based on diagnostic tests that have subsequently been found to be unsatisfactory under New Zealand conditions (McFadden and Heuer, 2011; McFadden, 2010).

In 2001 the IgA-ELISA was used to diagnose *Cfv* using vaginal mucus samples from cows (Hughes, 2001). However, a study by McFadden and others (2005) found no relationship between herd reproductive performance and the likelihood of a cattle herd testing positive with the IgA-ELISA. In addition, preputial washings from 54 bulls, selected for sampling from herds in which IgA antibody-positive cows had been detected, were negative when cultured for *Cfv*. The authors concluded that the specificity of the test was unsatisfactory under New Zealand conditions and that the IgA-ELISA is an unreliable test for detection of *Cfv* in New Zealand beef herds.

In 2007, a PCR (McMillen et al, 2006) was developed and offered commercially in New Zealand as a test for *Cfv* (Tisdall and Hill, 2007); however, suspicions were raised regarding the specificity of this test as a number of positive results were obtained from high fertility herds (Mcfadden and Heuer, 2011). In addition Hughes and others (2008) were unable to culture *Cfv* from samples collected from *Cfv* PCR positive animals including positive bulls, and heifers and cows mated to PCR positive bulls.

In 2009 *Campylobacter*-like organisms were cultured from the faeces of one bull on two occasions (Spence et al, 2011). The isolates were presumptively identified as *Cfv* based on biochemical test results and McMillen-PCR analysis. However, subsequent molecular testing showed that the isolate presumptively identified as *Cfv* was *Campylobacter hyointestinalis*, a commensal of the bovine gastro-intestine not known to cause adverse effects on reproductive performance (Spense et al, 2011).

In summary, reported detections of *Cfv* in New Zealand have relied on tests which have now been discredited. Hence, the only evidence for *Cfv* being present in New Zealand is a small number of historic reports of positive cultures. Given the issues with misclassification of *Cfv* and *Cff* it is possible that even these isolates are not truly *Cfv*.

The subspecies *Campylobacter fetus* subspecies *fetus (Cff)* is present in New Zealand (Mannering et al, 2004; Mannering et al, 2003) and is therefore not considered further in this risk analysis.

6.1.4 Epidemiology

Distribution of disease

Bovine genital campylobacteriosis, also known as bovine venereal campylobacteriosis, has a wide global distribution. The prevalence of disease appears to vary between countries, with highest disease prevalence observed in developing countries where natural breeding in cattle is widely practiced and lower prevalence of disease observed in developed countries where natural service is for the most part limited to beef herds (Mishelia et al, 2010).

Pathogenesis

Campylobacter fetus subsp. *venerealis* is highly adapted to the bovine genital tract and is regarded as an exclusive venereal pathogen. Infected bulls are the natural reservoir of infection for *Cfv*. The pathogen has been isolated from the glans penis, prepuce and the distal portion of the urethra (Eaglesome et al, 1995). *Cfv* colonizes the epithelial surface of the lumen and crypts of the prepuce and penis (Samuelson et al, 1966) and semen is contaminated during ejaculation. In heifers and cows, the sites of infection are within the lumen of the vagina, cervix, uterus and oviducts (Garcia et al, 1983).

Transmission

Campylobacter fetus subsp. *venerealis* is a venereal disease of cattle. Thus, transmission of the pathogen occurs primarily by coitus. Female cattle may become infected after being covered by an infected bull. The duration of infection in female animals varies and some females can remain carriers of the pathogen for up to two years. Non-infected bulls become infected when breeding an infected female and may remain persistently infected.

Transmission may also occur through artificial insemination (AI) using infected semen or through contaminated insemination equipment (Mishelia et al, 2010; Eaglesome et al, 1995; Garcia et al, 1983). Direct female to female spread is unlikely; however, bull to bull transmission has been suspected among bulls housed together where mounting behaviour is active (Hoffer, 1981; Clark, 1971).

Clinical signs

Campylobacter fetus subsp. *venerealis* infection in bulls is asymptomatic and is not associated with gross lesions, histological changes or altered semen quality (Eaglesome et al, 1995; Bier et al, 1977). Infection of susceptible heifers and cows is associated with varying grades of genital inflammation, including vaginitis, cervicitis, endometritis, and salpingitis (Schurig et al, 1974) and is characterised by lowered fertility, embryo mortality, abortion (may occur at any time but are most commonly detected at 4-6 months of gestation), repeated returns to service, reduced pregnancy rates and extended calving intervals (Mishelia et al, 2010).

Immune response

The persistent nature of infection with *Cfv* in bulls indicates that the pathogen can inhabit the lower genital tract and thrive in the absence of an effective specific antibody response (Vasquez et al, 1983; Bier et al, 1977; Samuelson and Winter 1966). In contrast to bulls, genital infection with *Cfv* in cows induces a detectable antibody response in the lower genital tract (Hum et al, 1991; Corbeil et al, 1974a; Corbeil et al, 1974b).

The duration of genital infection with *Cfv* in the uterus and uterine tubes of cows and heifers is generally limited to weeks or months but infection can persist for longer periods, 6-18 months (up to 24 months), in the vagina. Pathology is most pronounced 8-13 weeks after infection and has generally resolved within 4-5 months (Mishelia et al, 2007). Disease is generally self-limiting in females and most cases will recover and conceive within 3-6 months post infection. Immunity following recovery persists for variable periods and wanes over time (Truyers et al, 2014).

Diagnosis

Diagnosis of *Cfv* may be based on isolation and identification of the agent from the preputial smegma and semen of males, the cervicovaginal mucus in females or the placenta, liver, lungs and stomach contents of aborted foetuses. Methods of detection of the agent include culture, immunofluorescence antibody test (IFAT) or Monoclonal antibody-based capture ELISA (MAb-based capture ELISA) followed by culture. Confirmation of the isolate and discrimination between subspecies can be performed by biochemical or molecular methods. Of all described molecular tests, only polymerase chain reaction (PCR) assays (targeting the *nahE* gene), multilocus sequence typing (MLST), and amplified fragment length polymorphism (AFLP) are able to identify *C. fetus* species reliably (OIE 2017).

Diagnosis may also be based on the detection of *Cfv* antibodies. ELISA and vaginal mucus agglutination tests (VMATs) are available and are used to detect antibodies in vaginal mucus. However, although both of these tests are useful as a herd screening method for *Campylobacter fetus* infection (Truyers et al, 2014), they are not suitable for diagnosis of the infection in individual animals (OIE 2017).

Vaccination

In some countries commercial inactivated vaccines are available. The vaccine must incorporate the different antigens associated with *C. fetus* (OIE 2017). Systemic vaccinations against *Cfv* in cattle have been demonstrated to confer genital protection (Clark et al, 1974; Clark et al, 1977; Clark et al, 1979; Clark et al, 1982). In infected herds, all breeding animals should be vaccinated twice prior to the breeding season. Bulls require two vaccine doses annually, because the vaccine may not always be effective in terminating established infections.

In non-infected herds, only the bulls are vaccinated annually, and this will be done twice a year (two doses with 21 days interval; two weeks before the start of the breeding season) (OIE, 2017a).

Treatment

Successful treatment of *Cfv* consisting of local and systemic antibiotic therapy in bulls less than three years of age has been reported. Streptomycin was the most commonly used antimicrobial for treatment of *Cfv*, although resistant strains of *Cfv* have been detected (Hanel et al, 2011), and the use of this drug in food producing animals has become restricted in some countries. Preputial infusion of 3.0g amoxicillin suspension has also been suggested as an appropriate treatment for infected bulls although (DAWR, 2009) culling of older infected bulls is generally recommended.

Response to treatment is generally poor in infected heifers, cows and older bulls and is therefore not recommended. Culling of these animals is considered the most appropriate method of disease management.

Control of *Cfv* in semen

Combinations of antibiotics have been recommended for the purpose of controlling *Cfv* in frozen bovine semen (Shin et al, 1988). A study by Shisong and others (1990) demonstrated that incubation at 35°C for 40 minutes with penicillin, streptomycin, lincomycin and spectinomycin was required to reduce the numbers of *Cfv* in artificially contaminated semen to non-detectable levels in all samples tested.

A study by Hanel and others (2011) investigated the *in-vitro* susceptibility of *Cfv,* isolated from bovine specimens, to antibiotics generally used in semen treatment. The susceptibility of 50 isolates of *Cfv* to 8 different antibiotic treatments (Spectinomycin, gentamicin, streptomycin, penicillin, lincomycin, ciprofloxacin, erythromycin and tetracycline) was determined using a disk diffusion susceptibility test. The study concluded that all strains were susceptible to gentamicin. Seven of fifty (14%) showed considerably reduced susceptibility to one or more antimicrobial agents, with the most frequent reduction in susceptibility being to lincomycin and spectinomycin.

6.1.5 Hazard identification conclusion

Campylobacter fetus subsp. *venerealis* is the causative agent of BGC, an OIE listed disease which results in abortion and temporary infertility in infected heifers and cows. The organism has been isolated from the semen of infected bulls and the reproductive tract of infected heifers and cows. *Cfv* is identified as a hazard in the commodity.

RISK ASSESSMENT

6.2.1 Entry assessment

Semen

Campylobacter fetus subsp. *venerealis* is a persistent infection in bulls, and is not associated with clinical signs, gross lesions or altered semen quality. It is therefore plausible that infected bulls may be used as semen donors. *Campylobacter fetus* subsp. *venerealis* colonises the epithelial surface of the lumen and crypts of the prepuce and penis and semen is contaminated during ejaculation.

The likelihood of entry is assessed to be high.

In-vivo derived embryos

Campylobacter fetus subsp. venerealis has been isolated from the lumen of the vagina, cervix, uterus and oviducts of infected females.

Given that *in-vivo* derived (IVD) embryos are collected from within the uterus it is plausible that embryos could be contaminated through exposure to *Cfv* along the reproductive tract.

Whether or not viable *Cfv* may persist in IVD embryos which have been subjected to standard collection and processing protocols does not appear to have been studied.

The likelihood of entry is therefore assessed to be non-negligible for IVD embryos prepared in accordance with the IETS Manual.

In-vitro produced embryos

In-vitro produced (IVP) embryos are produced from oocytes collected from the ovaries of donor cows. The ovaries, unlike the uterus, have not been reported as a site of infection for *Cfv*. Therefore, it is unlikely that collected oocytes and resultant IVP embryos would be contaminated with *Cfv*.

Furthermore, IVP embryos have been shown to be free from viable *Cfv* when standard processing recommendations set out by the IETS have been carried out (Bielanski et al, 1994; Guerin et al, 1988).

Thus, the likelihood of entry is assessed to be negligible for IVP embryos prepared in accordance with the IETS Manual.

6.2.2 Exposure assessment

Semen

The likelihood of exposure is certain since imported semen is inseminated into susceptible females. Venereal transmission of *C. fetus* subsp. *venerealis* via bovine semen, by natural service and artificial insemination, is well documented (Garcia et al, 1983; Hoffer 1981). The likelihood of transmission should *Cfv* be present in semen is high.

The likelihood of exposure is therefore assessed to be high.

In-vivo derived embryos

The OIE Code Chapters 4.7 and 11.3 do not recommend any specific measures for bovine campylobacteriosis for international trade in IVD embryo. In addition, there is no evidence in the published literature demonstrating that viable *Cfv* persists in IVD embryos which have been subjected to standard collection and processing protocols set out in the Code Chapter 4.7.5. There is also no published studies to prove that infected *Cfv* embryos contain an infectious dose that would initiate an infection in the recipient female.

Thus, the likelihood of exposure is assessed to be negligible.

6.2.3 Consequence assessment

Bovine genital campylobacteriosis decreases animal productivity through early embryo losses, repeat breeding and increased calving intervals. It is an OIE listed disease that causes significant economic losses as a result of compromised animal productivity. In Argentina, weaning rates in BGC-infected herds decrease by 10%, which accounts for an annual loss of $165 million (Jimenez et al, 2011).

Other economic impacts include both loss of genetic potential and increased costs of replacements due to culling. It has been estimated that a reduction in gross profit margin of 66% and 36% may be observed in newly infected herds and herds in which the agent has become established, compared to uninfected herds (Hum et al, 1994).

In New Zealand, the vast majority of dairy calves are born to AI. Therefore the economic losses due to BGC would be mostly linked to the beef cattle sector where natural mating is used. The economic impact is assessed to be non-negligible.

Only cattle (*B.indicus* and *B. taurus*) are reported to be infected with *Cfv.* The impact to wildlife and the environment is assessed to be negligible.

Campylobacter fetus subspecies *venerealis* is restricted to the genital tract of cattle. There is just a single report of a human case relating to the isolation of *Cfv* from a woman with bacterial vaginosis in Sweden in 1987 (Holst et al, 1987). *Campylobacter fetus* subspecies *venerealis* is therefore not considered a zoonotic pathogen and impacts to human health are assessed to be negligible.

6.2.4 Risk estimation

Since the entry, exposure and consequence assessments for *Cfv* in semen are non-negligible, *Cfv* is assessed to be a risk in imported semen. Consequently, risk management measures are scientifically justifiable.

Since the likelihood of entry of *Cfv* in IETS processed IVD and IVP embryos is assessed to be negligible, the risk estimate for embryos is assessed to be negligible.

Accordingly risk management measures are not required for *Cfv* in IVD and IVP embryos.

6.3 RISK MANAGEMENT

Embryos that meet the commodity definition (processed in accordance with IETS recommendations), require no additional risk management since the risk estimate is negligible.

This section will not discuss embryos further, but focuses on managing the risk of introducing *Cfv* associated with semen.

Accordingly, the following points were taken into account when describing options for effectively managing the risk in semen.

- Bovine genital campylobacteriosis (BGC) is an OIE listed disease (OIE 2018).
- *Campylobacter fetus* subsp. *venerealis*, the causative agent of BGC is notifiable under the Biosecurity (Notifiable Organisms) Order 2016.
- There has been no laboratory confirmed isolation of the agent since 1992.
- *Campylobacter fetus* subsp. *venerealis* is a venereally transmitted pathogen through natural service or AI using infected bull semen.
- Clinically healthy bulls may be infected since infection in bulls is not associated with clinical signs, gross lesions, histological changes or altered semen quality.
- In view of this, the OIE recommends testing of bulls by culture for *Cfv* to demonstrate freedom from infection. Other diagnostic methods include immunofluorescence and monoclonal antibody-based capture ELISA.
- The *Code* Chapter 4.6 provides general recommendations for hygienic collection and processing of semen.
- The *Code* Chapters 4.6 and 11.3 make specific recommendations for *Cfv* to ensure the safe international trade of semen.

Options

Option 1

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

1. the donor animals:
    a. have never been used for natural service; or
    b. have only mated virgin heifers; or
    c. were kept in an artificial insemination centre where no case of bovine genital campylobacteriosis has been reported;

And

2. the culture of semen and preputial specimens for the presence of the causal agent of bovine genital campylobacteriosis proved negative.

N.B. This option aligns with the recommendations provided by *Code* Chapter 11.3. Some of the included sub-options may be difficult to certify. This option should only be considered for countries where BGC is a notifiable disease.

Option 2

The semen was collected, processed and stored in accordance with Chapters 4.5. and 4.6. of the *Code*;

- The donor animal has been kept in a pre-entry isolation facility for at least 28 days and after a minimum of 7 days in pre-entry isolation has undergone testing for *Campylobacter fetus* subspecies *venerealis* as follows:
    I. Animals less than six months old or kept since that age only in a single sex group prior to pre-entry isolation should be tested once on a preputial specimen, with a negative result.
    II. Animals aged six months or older that could have had contact with females prior to pre-entry isolation should be tested three times at weekly intervals on a preputial specimen, with a negative result in each case.

And

- All resident bulls and teasers in the semen collection facility are tested at least annually for *Campylobacter fetus* subspecies *venerealis*, with negative results, where the country or zone where the semen collection facilities are located is not free
    I. A preputial specimen should be tested.
    II. Only bulls on semen production or having contact with bulls on semen production need to be tested. Bulls returning to collection after a lay-off of more than six months should be tested not more than 30 days prior to resuming production.

N.B. This is the current import health measure and aligns with the commodity definition and the recommendations for the importation of bovine semen provided by *Code* Chapter 4.6.

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