

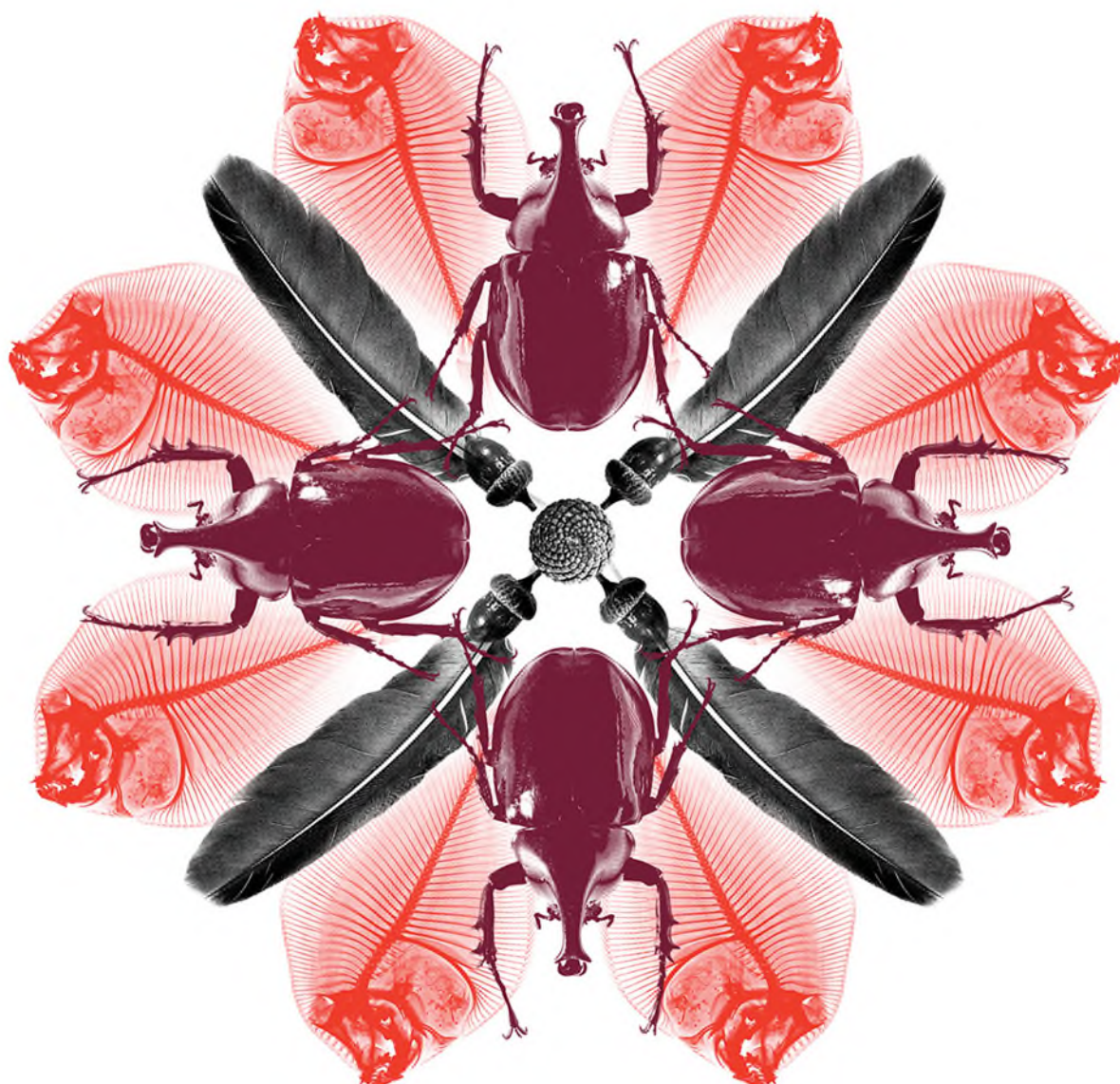


Australian Government
Department of Agriculture
and Water Resources

Importation of frozen bovine in vitro produced embryos from Canada and the United States – final review

March 2017

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Acronyms and abbreviations

Term or abbreviation	Definition
AGID	agar gel immunodiffusion
AHA	Animal Health Australia
ALOP	appropriate level of protection
AQPM	Animal Quarantine Policy Memorandum
BGC	bovine genital campylobacteriosis
BIRA	Biosecurity Import Risk Analysis
BLV	bovine leukaemia virus
BoHV	bovine herpesvirus
BSE	bovine spongiform encephalopathy
BT	bluetongue
BTV	bluetongue virus
BVD	bovine viral diarrhoea
BVDV	bovine pestivirus (also known as bovine viral diarrhoea virus)
CBPP	contagious bovine pleuropneumonia
cELISA	competitive enzyme linked immunosorbent assay
CFIA	Canadian Food Inspection Agency
CFT	complement fixation test
COCs	cumulus-oocyte complexes
CP	cytopathic
DNA	deoxyribonucleic acid
EAD	emergency animal disease
EADRA	Emergency Animal Disease Response Agreement
EBL	enzootic bovine leucosis
EHD	epizootic haemorrhagic disease of deer
EHDV	epizootic haemorrhagic disease virus
ELISA	enzyme linked immunosorbent assay
FMD	foot and mouth disease
IETS	International Embryo Technology Society
IETS Manual	Manual of the International Embryo Technology Society
IBR/IPV	infectious bovine rhinotracheitis/ infectious pustular vulvovaginitis

Importation of frozen bovine IVP embryos

Term or abbreviation	Definition
IND	Indiana
JD	Johne's disease
LSD	lumpy skin disease
<i>Map</i>	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>
MAT	microscopic agglutination test
MD	mucosal disease
mL	millilitre
MLV	modified live virus
NCP	non-cytopathic
NJ	New Jersey
OIE	World Animal Health Organisation
OIE Code	OIE Terrestrial Animal Health Code
OIE Manual	OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
OPU	ovum pick-up
qRT-PCR	real-time quantitative reverse transcriptase polymerase chain reaction
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
RVF	Rift Valley fever
SBV	Schmallenberg virus
SC	small colony
SRMs	specified risk materials
sv / svs	serovar / serovars
TB	bovine tuberculosis
TCID ₅₀	50% tissue culture infective dose
USDA	United States Department of Agriculture
USDA: APHIS	USDA: Animal and Plant Health Inspection Services
VS	vesicular stomatitis
VSV	vesicular stomatitis virus

Summary

This review, undertaken by the Australian Government Department of Agriculture and Water Resources, evaluates the biosecurity risks for Australia associated with the importation of frozen bovine in vitro produced embryos derived from live donors from Canada and the United States.

Australia has for many years imported bovine semen and in vivo derived embryos from both countries. The previous major reviews of biosecurity requirements for the importation of bovine germplasm (semen and both in vivo derived and in vitro produced embryos) were conducted in 1993 for Canada and 1997 for the United States. These reviews established biosecurity requirements for semen and in vivo derived embryos from these countries but not for in vitro produced embryos due to the complexities of the in vitro processes at the time.

However, recent scientific advances in reproductive technology have led to requests for Australia to review the biosecurity risks associated with importing in vitro produced embryos.

This review concluded that risk management was necessary for each of the following four stages of the in vitro produced embryo processing system in order to ensure that Australia's appropriate level of protection (ALOP) is achieved:

- 1) the health status of both oocyte donors and semen
- 2) the sanitary collection of oocytes
- 3) the sanitary in vitro processing of oocytes through to blastocyst stage
- 4) the effective freezing and storage of in vitro produced embryos.

For the health status of both oocyte donors and the semen used for in vitro fertilisation, the review concludes that biosecurity measures be applied to manage the following diseases: infection due to Schmallenberg virus, bluetongue, bovine viral diarrhoea, enzootic bovine leucosis, epizootic haemorrhagic disease, foot and mouth disease, infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (bovine herpesvirus type 1), lumpy skin disease, Rift Valley fever, vesicular stomatitis, brucellosis (*Brucella abortus* and *B. melitensis*), bovine tuberculosis (*Mycobacterium bovis*), and contagious bovine pleuropneumonia (*Mycoplasma mycoides* subsp. *mycoides* SC).

Table 1 provides a summary of the recommended biosecurity measures for each of these diseases of biosecurity concern. These measures apply only to frozen bovine in vitro produced embryos derived from live donors and imported from Canada and the United States. Full details of the review and conclusions for each disease are provided in Chapter 4. These measures are detailed in the import conditions provided in Chapters 5 and 6.

Table 1 Summary of the biosecurity measures for each disease of biosecurity concern

Refined Hazards	Biosecurity measures
VIRAL DISEASES	
Infection due to Schmallenberg virus	No cases of disease caused by Schmallenberg virus have been detected or reported in Canada/the United States
Bluetongue	Certify either country freedom to OIE Code standards or diagnostic test
Bovine viral diarrhoea	Certify diagnostic tests results to show no viraemia or persistent infection in donors and washing embryos to IETS Manual standards
Enzootic bovine leucosis	Certify semen from donors free from bovine leukaemia virus to OIE Code and washing embryos to IETS Manual standards
Epizootic haemorrhagic disease	Certify either country freedom to OIE Code standards or diagnostic test
Foot and mouth disease	Certify country freedom as recognised by the OIE and Australia
IBR/IPV - Bovine herpesvirus-1	Certify herd freedom to OIE Code standards, or diagnostic test and washing embryos to IETS Manual standards
Lumpy skin disease	Certify country freedom to OIE Code standards
Rift Valley fever	Certify country freedom to OIE Code standards
Vesicular Stomatitis	Certify premises freedom
BACTERIAL DISEASES	
Bovine brucellosis (<i>B. abortus</i>)	Certify country/zone freedom and herd free without vaccination to OIE Code standards
Brucellosis due to <i>B. melitensis</i>	Certify country freedom to OIE Code standards
Bovine tuberculosis	Certify country/zone freedom and herd free to OIE Code standards
Contagious bovine pleuropneumonia	Certify country freedom as recognised by the OIE

For the remaining three stages of the in vitro produced embryo processing system, the review recommends that the standards as recommended by the World Animal Health Organisation Terrestrial Animal Health Code (OIE Code) and the Manual of the International Embryo Technology Society (IETS Manual) apply to the sanitary collection of oocytes through to freezing and storage of in vitro produced embryos. The articles relevant to live oocyte donors in Chapters 4.8 and 4.9 of the OIE Code are the minimal standards that apply for the importation of frozen bovine in vitro produced embryos. These standards determine the certifiable conditions for:

- 1) the embryo production team (EPT) and approved EPT veterinarian
- 2) the processing laboratories involved
- 3) the donor animals (male for semen and female for oocytes)
- 4) embryo treatments (e.g. antibiotics, biological product of animal origin) and equipment used in the processes
- 5) the storage and transport of the in vitro produced embryos
- 6) the micromanipulation of in vitro produced embryos.

1 Introduction

1.1 Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, agricultural industries that are relatively free from serious pests and diseases, and human health.

The risk analysis process is an important part of Australia's biosecurity policies. It enables the Australian Government to formally consider the level of biosecurity risk that may be associated with proposals to import goods into Australia. If the biosecurity risks do not achieve the appropriate level of protection (ALOP) for Australia, risk management measures are recommended to reduce the risks to an acceptable level. If the risks cannot be reduced to an acceptable level, the goods will not be imported into Australia, until suitable measures are identified.

Successive Australian Governments have maintained a conservative, but not a zero risk, approach to the management of biosecurity risks. This approach is expressed in terms of Australia's ALOP, which reflects community expectations through government policy and is currently described as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

Australia's risk analyses are undertaken by the Australian Government Department of Agriculture and Water Resources using technical and scientific experts in relevant fields, and involve consultation with stakeholders at various stages during the process.

Risk analyses may take the form of a biosecurity import risk analysis (BIRA) or a non-regulated risk analysis (such as scientific review of existing policy and import conditions, or scientific advice).

Further information about Australia's biosecurity framework is provided in the *Biosecurity Import Risk Analysis Guidelines 2016* located on the [Australian Government Department of Agriculture and Water Resources website](https://agriculture.gov.au/biosecurity/risk-analysis/guidelines) (agriculture.gov.au/biosecurity/risk-analysis/guidelines).

The Department of Agriculture and Water Resources recognises that there might be new scientific information and technologies, or other combinations of measures that may provide an equivalent level of biosecurity protection for the disease agents identified as requiring risk management. Submissions supporting equivalence measures will be considered on a case-by-case basis.

1.2 This review

1.2.1 Background

This review of biosecurity risks associated with the importation into Australia of frozen bovine in vitro produced embryos has been undertaken in response to requests by stakeholders to

expand the options for importing bovine genetics beyond the current conditions allowing the importation of frozen bovine semen and frozen bovine in vivo derived embryos.

Biosecurity requirements currently exist for the importation into Australia of bovine semen and in vivo derived embryos from Canada, the Member States of the European Union, New Caledonia, New Zealand, Norway, Switzerland and the United States. There were biosecurity policies for frozen bovine in vivo derived embryos from Zimbabwe from 1999 until suspension in 2001 and the Republic of South Africa from 1997 until suspension in 2011. Both suspensions were due to outbreaks of foot and mouth disease. Conditions also exist for the importation of frozen bovine in vitro produced embryos from New Zealand.

Australia has not permitted import of live cattle for some years due to bovine spongiform encephalopathy or bovine tuberculosis being reported in countries that previously could export to Australia. Given the advances in artificial breeding technology and the growing acceptance of safe international trade in bovine germplasm, it is unlikely there will be much demand for importation of live cattle in the near future.

The previous major reviews of biosecurity requirements for the importation of germplasm (semen and both in vivo derived and in vitro produced embryos from Canada and the United States) were conducted in 1993 and 1997. The outcome of the 1997 review was biosecurity requirements for bovine semen and in vivo derived embryos not subjected to micromanipulation. The review concluded that due to complexities in the in vitro produced embryo process, the biosecurity requirements for bovine in vivo derived embryos could not apply to bovine in vitro produced embryos.

Although in vitro produced embryo production is a relatively new reproductive technology tool in the livestock industry, it has grown rapidly in recent years due to its acceptance by the cattle breeders as a valuable and economic tool for improving cattle genetics and hence cattle production. Since the first calf was born after in vitro fertilisation in 1982, ongoing developments in oocyte collection in live cows and heifers have resulted in successful commercial application of this technology in many countries. These developments include the introduction of ultrasound transvaginal guided techniques for recovering oocytes from live donor cows around 1998, reproducible laboratory techniques for in vitro maturation of oocytes, in vitro fertilisation, in vitro culture, micro-manipulation to collect DNA for genetic/genomic evaluation, and freezing of in vitro produced embryos.

Table 2 shows the known number of bovine in vitro produced embryo collected by ovum pick-up (OPU) world-wide in 2014 and 2015 (Perry 2016).

Table 2 Collection and transfer of OPU bovine in vitro produced embryos by region

Region	OVUM PICK UP									
	Collection			Transfers		Collection			Transfers	
	Donors	Oocytes	Embryos	Fresh	Frozen	Donors	Oocytes	Embryos	Fresh	Frozen
	2014					2015				
Africa	1359	20976	5081	1202	170	1113	21494	3733	162	21
Asia	0	0	0	0	0	3177	59224	9438	3250	1164
Europe	9710	83785	15693	10980	2957	9092	73397	13780	8115	4345
North America	43452	812468	206139	71263	21667	35980	683717	212046	65844	32027
Oceania	3250	30549	6486	2044	3171	1646	17533	3892	1825	2491
South America	71327	861100	356960	211177	40096	60696	1205840	369820	224066	58821
Grand Total	129098	1808878	590359	296666	68061	111704	2061205	612709	303262	98869

Although sanitary conditions related to in vivo derived embryos have been extensively studied and applied, these results are not necessarily applicable to in vitro produced embryos. In reviewing the biosecurity risks for bovine in vitro produced embryos, it is necessary to evaluate the in vitro produced process, from the donor animals (bulls for semen and cows for oocytes) through to export of in vitro produced embryos when developing sanitary conditions for in vitro produced embryos (Perry 2007). The issues that influence the biosecurity risk include:

- collection of oocytes from live donors. Oocytes may be infected with pathogens as a result of infection in the donor female or be contaminated with pathogens during collection
- differences in the zona pellucida between in vivo derived and in vitro produced embryos, the zona pellucida generally being “stickier” for pathogens in in vitro produced embryos. Thus pathogens may not be removed by washing in vitro produced embryos, even with trypsin washes
- lack of set procedures or recommendation for allocating harvested oocytes into groups for the in vitro produced embryo process. Laboratories generally allocate the batch into groups based on individually identified donors, or in lots of 10, 20, 50, or even 100 cumulus-oocyte complexes (COCs). Using more than one donor in batches can result in spread of contamination from infected oocytes from infected donors to clean oocytes of healthy donors
- in vitro maturation process. The purpose of oocyte maturation is to resume the meiosis process of transforming the primary oocyte into a mature secondary oocyte or ovum receptive to fertilisation. The cumulus cells surrounding the oocytes are crucial to the in vitro maturation and in vitro fertilisation processes and may harbour pathogens
- lack of standards specifying regular washing of oocytes/embryos between stages during the in vitro process. The number of washings and their dilutions vary considerably among laboratories and affect the dilution and/or removal of pathogens not infecting or adhering to COCs. Washing three times between each stage is the recommended sanitary practice
- quality of bovine semen used for in vitro fertilisation stage, especially the source and health status of the semen donor. Although fresh semen can be used, frozen semen collected from a donor in a licensed semen collection centre is typically used for commercial production of in vitro produced embryos as this provides assurance that the health status of the bull was satisfactory and semen was collected hygienically
- standards specifying fertilisation of ova for the in vitro fertilisation process. There are different methods of separating out highly motile spermatozoa from seminal plasma, extender and/or cryoprotectants for fertilising the oocyte, e.g. the differential gradient centrifugation technique, swim-up and centrifugation technique or the simple washing procedure. Each of these methods require the addition of media free from pathogens and should be conducted under sanitary conditions
- addition of biologicals during in vitro maturation, in vitro fertilisation and in vitro culture. Fetal calf serum is a common ingredient in the various media used during the in vitro process, being commonly used in pre-in vitro maturation washes, in the in vitro maturation media, and in the in vitro culture media. Bovine serum albumin serves as a macromolecular

substitute in media for oocyte maturation, fertilisation and early embryo culture. It provides essential embryotrophic and sperm capacitation functions. The recommended sanitary practice for ensuring that biologicals presented no animal disease risk is that biologicals be certified by the manufacturer as free from pathogens

- preparation of zygotes for in vitro culture. Six to 18 hours after the beginning of in vitro fertilisation, zygotes are usually removed from the in vitro fertilisation medium, washed free of residual sperm and gently stripped naked of all cumulus cells before being transferred to the development medium for in vitro culture. It is important that zygotes be completely denuded prior to in vitro culture thus preventing any growth of contaminants or pathogens in cumulus cells during in vitro culture
- addition of antibiotics to the media to protect against growth of contaminant microorganisms, especially during in vitro culture
- use of somatic cells for in vitro culture. To culture zygotes to blastocyst stage, fertilised oocytes may be co-cultured with a suspension of bovine oviductal epithelial cells or other somatic cells or synthetic media mimicking these oviductal cells, and incubated in droplets for 7–8 days under oil at 39.8 °C in 5% CO₂ in humidified air. Although a wide variety of somatic cells can be used as co-culture cells in the in vitro culture phase, monolayers of co-culture cells prepared from the cumulus cells previously stripped from the oocytes prior to in vitro fertilisation is a common practice. Pathogens, if present, may adhere to or infect co-culture cells or contaminate the culture media. Pathogens infecting or contaminating the culture may grow and proliferate during in vitro culture, depending on resistance to antibiotics, presence of antibodies, and suitability of the culture system for pathogen growth within the in vitro culture timeframe
- use of micro-manipulation techniques for collecting DNA for genetic/genomic analysis to estimate the breeding value of an embryo. This involves breaching the zona pellucida, thus it is critical that sanitary and hygienic procedures apply to prevent contamination of the embryo
- freezing method used. Slow freezing does not involve direct contact with liquid nitrogen whereas some vitrification methods involve direct contact with liquid nitrogen. Thus the sterility of liquid nitrogen is important when used for vitrification to prevent contamination of embryos with pathogens.

All these issues are to be addressed so that risk of pathogens infecting or contaminating oocytes at all stages of the in vitro production process is negligible in order for in vitro produced embryo transfer to be a safe technology. The *OIE Terrestrial Animal Health Code* (OIE Code) and the *Manual of the International Embryo Technology Society* (IETS Manual) provide sound sanitary guidelines for the safe transfer of in vitro produced embryos as they do with in vivo derived embryos. However, both the OIE Code (OIE 2016f) and the IETS Manual (IETS 2010) consider the different stages of the in vitro produced embryo process as a single unit of operation. In reality, there can be up to four discrete commercial units of operation involved, each of which may operate independently or cooperatively, having its own team of people:

- 1) The semen collection centre—which affects the health status of semen used for in vitro fertilisation.

- 2) The oocyte collection centre—which affects the health status of oocyte donors, sanitary collection of immature oocytes and the start of the in vitro maturation process.
- 3) The in vitro produced embryo processing laboratory—where the sanitary in vitro processing of mature oocytes through to blastocyst stage, including in vitro fertilisation, and freezing are conducted.
- 4) The germplasm storage centre—where in vitro produced embryos are stored prior to export.

The import conditions take into account situations where there may be up to four discrete commercial units of operations involved in the whole process.

In addition, the OIE Code Chapter 4.8 recommends the embryo production team, defined as a group of competent technicians, including at least one veterinarian, to perform the collection and processing of oocytes and the production and storage of in vitro produced embryos, be approved by the competent authority (OIE 2016f). For export of in vitro produced embryos, Canada and the United States approve the embryo production team and/or the Team Veterinarian who is first certified by the respective national embryo transfer associations as competent in the practice of in vitro produced embryo transfer. The Team Veterinarian has the responsibility of ensuring that the embryo production team is competent as defined by the OIE Code.

The *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (OIE Manual) provides recommendations and guidelines for minimal standards to apply to diagnostic tests and vaccines.

1.2.2 Scope

This review assesses the biosecurity risks associated with the importation of frozen bovine in vitro produced embryos imported from Canada and the United States. The oocytes used for producing in vitro produced embryos are to be collected surgically, e.g. ovum pickup technique, from live donor cows or heifers, not from ovaries of cattle at abattoirs or dead animals.

Eligible embryos include those subject to micromanipulation only for the purpose of collecting biopsy samples for genetic analysis.

The standards used for collection, processing, freezing and storage of bovine in vitro produced embryos are the OIE Code and the IETS Manual.

1.2.3 Existing policies

Import policies

Bovine germplasm has been imported into Australia under conditions set for the importation of bovine semen and in vivo derived embryo from Canada, member states of the European Union, New Caledonia, New Zealand, Norway, Switzerland and the United States. Conditions also exist for the importation of frozen bovine in vitro produced embryos from New Zealand.

Imports of germplasm are restricted to these countries as they provide a satisfactory level of assurance for certifying to Australia's biosecurity requirements.

These conditions require risk management for the following hazards that may be present in the approved countries and assessed to be above Australia's appropriate level of protection (ALOP).

Viral diseases

- Infection due to Schmallenberg virus, bluetongue, bovine viral diarrhoea, enzootic bovine leucosis, epizootic haemorrhagic disease, foot and mouth disease, IBR/IPV (bovine herpesvirus type 1), lumpy skin disease, Rift Valley fever, vesicular stomatitis.

Bacterial diseases

- Brucellosis (*Brucella abortus*, *B. melitensis*, *B. suis*), paratuberculosis (*Mycobacterium avium* subsp. *paratuberculosis*), bovine genital campylobacteriosis (*Campylobacter fetus* subsp. *venerealis*) (semen only), bovine tuberculosis (*Mycobacterium bovis*), contagious bovine pleuropneumonia (*Mycoplasma mycoides* subsp. *mycoides* SC).

Protozoal diseases

- Trichomonosis (*Tritrichomonas foetus*) (semen only).

Existing (and recommended) risk management measures include that bovine semen be collected and processed according to the OIE Code Chapters 4.5 and 4.6, and the in vivo derived embryos collected and processed according to the OIE Code Chapters 4.7. Micromanipulated embryos were not permitted to be imported. With regards to importing in vitro produced embryos from New Zealand, the conditions were based on the conditions for importing live cattle from New Zealand; bovine tuberculosis being the main disease present in New Zealand and of concern to Australia. Conditions for importation of live cattle from New Zealand were originally promulgated on August 1997 but suspended after it was realised that there were difficulties in meeting the certification requirement for bovine tuberculosis.

The full import requirements for bovine semen and bovine in vivo derived embryos can be found on the department's [Biosecurity Import Conditions System \(BICON\) website](https://agriculture.gov.au/import/online-services/bicon) (agriculture.gov.au/import/online-services/bicon).

Domestic arrangements

The Australian Government is responsible for regulating the movement of animals and their products into and out of Australia. The state and territory governments have primary responsibility for animal health and environmental controls within their jurisdictions. Legislation may be used by state and territory governments to control interstate movement of animals and their products. Once animals and animal products have been cleared by Australian biosecurity officers, they may be subject to interstate movement controls.

Currently, there are no restrictions to the interstate movements of bovine semen and embryos. However, it is the importer's responsibility to identify and ensure compliance with all requirements.

1.2.4 Next Steps

Stakeholders were given the opportunity to comment on technical aspects of the proposed biosecurity measures. In particular, comments were sought on the appropriateness of the measures or alternative measures that would provide equivalent risk management measures. The department considered formal submissions on this review. The department then prepared a final report taking into account stakeholder comments.

The final review is published on the department's website along with a notice advising stakeholders of the release. The department will work with Canada and the United States to finalise sample animal health certificates so that trade in bovine in vitro produced embryos can commence.

2 Method

The method used in the evaluation of the risk management measures involved in the importation of frozen bovine in vitro produced embryos from Canada and the United States is based on the principles of the OIE Code Chapter 2.1. This method is the same as that described in Chapter 2 of the *Import risk analysis report for horses from approved countries: final policy review* (Department of Agriculture, Fisheries and Forestry 2013) and also Chapter 2 of the *Importation of dogs and cats and their semen from approved countries: final policy review* (Department of Agriculture 2013).

2.1 Background

The OIE Code (OIE 2016f) describes 'General obligations related to certification' in Chapter 5.1.

Article 5.1.2 of the OIE Code states that:

The import requirements included in the international veterinary certificate should assure that commodities introduced into the importing country comply with the standards of the OIE. Importing countries should align their requirements with the recommendations in the relevant standards of the OIE. If there are no such recommendations or if the country chooses a level of protection requiring measures more stringent than the standards of the OIE, these should be based on an import risk analysis.

Article 5.1.2 further states that:

The international veterinary certificate should not include measures against pathogens or diseases which are not OIE listed, unless the importing country has demonstrated through import risk analysis, carried out in accordance with Section 2, that the pathogen or disease poses a significant risk to the importing country.

The components of risk analysis as described in Chapter 2.1 of the OIE Code are:

- hazard identification
- risk assessment (entry assessment, exposure assessment, consequence assessment and risk estimation)
- risk management
- risk communication.

Hazard identification, risk assessment and risk management are sequential steps within the risk analysis process. Risk communication is conducted as an ongoing process, and includes both formal and informal consultation with stakeholders.

2.2 Risk review

Although not defined or described in the OIE Code, risk review is recognised by risk analysts as an essential component of the risk analysis process (Barry 2007; Brett, Rodricks & Chinchilli 1989; FSA 2006).

Australia applies a process of risk review to the biosecurity risks associated with the importation of an animal commodity (animal product or live animal) for which biosecurity measures currently apply.

Risk review differs from the monitoring and review component of risk management, as described in the OIE Code, in that each component of the BIRA process (hazard identification, risk assessment and risk management) is reviewed under the risk review process. If a change (either an increase or a decrease) in the biosecurity risk associated with a live animal or animal product that is currently imported into Australia is identified based on updated technical information, risk management measures can be revised accordingly.

This review has drawn on many sources of information including:

- the OIE Terrestrial Animal Health Code (OIE 2016f)
- Manual of the International Embryo Technology Society (IETS 2010)
- the Department of Agriculture and Water Resources
 - Import Risk Analysis Report on the Importation of Bovine Semen and Embryos from Argentina and Brazil into Australia (AQPM 2000/3)
 - An Analysis of the Disease Risks, other than Scrapie, associated with the Importation of Ovine and Caprine Semen and Embryos from Canada, The United States of America and Member States of the European Union (AQPM 2000/38)
 - Import conditions for the importation of bovine embryos from the Republic of South Africa (suspended on 04 March 2011)
 - Current import conditions for the importation of bovine semen and in vivo derived embryos from Canada, the Member States of the European Union, New Caledonia, Norway, Switzerland and the United States of America (<https://bicon.agriculture.gov.au/BiconWeb4.0>)
 - Current import conditions for the importation of bovine semen and in vivo derived and in vitro produced embryos from New Zealand (<https://bicon.agriculture.gov.au/BiconWeb4.0>)
 - Technical Issues Paper for the bovine, ovine and caprine semen from the Republic of South Africa import risk analysis (AQPM 2002/30)
- Review of quarantine disease risks related to bovine semen: a report for the Australian Quarantine and Inspection Service (Adams 1995)
- a review of relevant scientific literature.

Risk, defined by the OIE Code as ‘the likelihood of the occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal or human health’ (OIE 2016f), is dynamic in nature; it changes with time. Consequently, risk should be kept under regular review.

2.3 Review of hazard identification

Hazard identification is described in the OIE Code (Article 2.1.2) as a classification step that is undertaken to identify potential hazards that may be associated with the importation of a commodity (OIE 2016f).

For this review, and in accordance with the OIE Code, a disease agent was determined to be a potential hazard relevant to the importation of frozen bovine in vitro produced embryos if it was assessed to be:

- appropriate to the species being imported
- OIE-listed, emerging and/or capable of producing adverse consequences in Australia.

A hazard was retained for further review (hazard refinement) if:

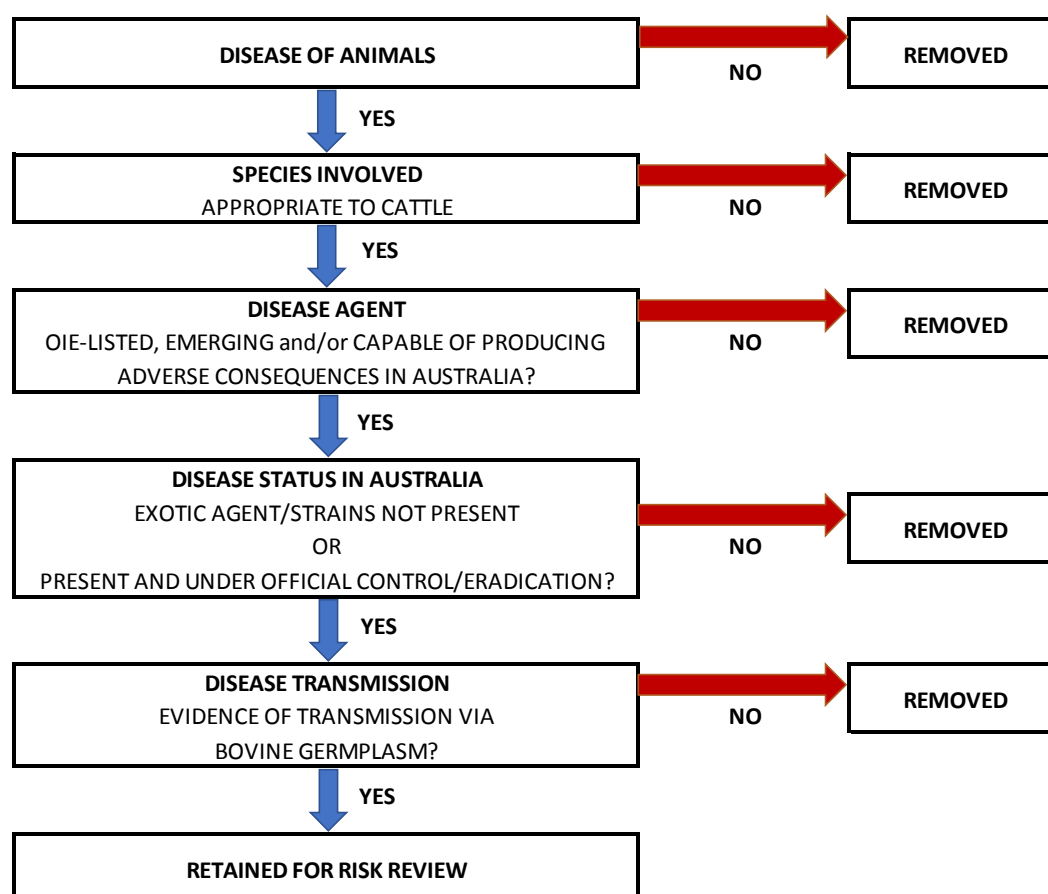
- it was not present in Australia, or present in Australia and subject to official control or eradication
and
- there was clear evidence of transmission via bovine germplasm (semen and embryos, both in vivo derived and in vitro produced).

This review considered the potential hazards identified in past import risk analyses. Evaluation of the current scientific literature was conducted to determine if hazards identified in these reports should be retained for further consideration and whether additional hazards should be added.

Where evidence for the inclusion or exclusion of a particular disease agent was equivocal, a judgement was made based on the strength of the available evidence to implicate bovine in vitro produced embryos in disease transmission.

In addition, all disease agents for which biosecurity measures applied under the Department of Agriculture and Water Resources conditions for the importation of bovine germplasm (semen and embryos, both in vivo derived and in vitro produced) were included and retained for further review.

The steps involved in hazard identification and refinement are shown in Figure 1.

Figure 1 Decision tree for hazard identification and refinement

2.4 Review of risk assessment

For each hazard retained for further assessment, a review of the scientific literature was performed to identify evidence of a significant change in the risk factors relevant to the release, exposure and consequence assessment of the hazard that would be relevant to biosecurity considerations for Australia. The advice of experts with specialist knowledge of disease agents was also obtained in some instances.

Uncertainties not resolved through literature review or contact with relevant experts were identified and documented.

Based on the information reviewed, a conclusion was made for each hazard regarding whether a significant change in biosecurity risk had occurred that was relevant to the importation of frozen bovine in vitro produced embryos into Australia. Assumptions and judgements made in drawing conclusions for each hazard retained for further review are documented in the relevant risk review section of Chapter 3.

2.5 Review of risk management

The OIE Code (Chapter 2.1) divides risk management into four processes:

- 1) Risk evaluation—the process of comparing the risk estimated in the risk assessment with the member's ALOP.

Australia's ALOP has not changed significantly since the last major review of the import conditions for bovine germplasm. The conclusions drawn from the risk reviews conducted for each hazard were used as the basis for risk evaluation. A judgement was then made to determine whether risk management was warranted to achieve Australia's ALOP. This method was considered to be appropriate to evaluate the biosecurity risks associated with the previous policy for the importation of bovine germplasm into Australia.

- 2) Option evaluation—the process of identifying, evaluating the efficacy and feasibility of, and selecting measures to reduce the risk associated with an importation to bring it in line with the member's ALOP.

Efficacy is the degree to which an option reduces the likelihood and/or magnitude of adverse health and economic consequences. Evaluating the efficacy of the options selected is an iterative process that involves their incorporation into the risk assessment and then comparing the resulting level of risk with that considered acceptable. The evaluation for feasibility normally focuses on technical, operational and economic factors affecting the implementation of the risk management options. In this review, reviews of risk management options for each hazard retained for further assessment were also undertaken and documented in the relevant risk review section (Chapter 3).

- 3) Implementation—the process of following through with the risk management decision and ensuring that the risk management measures are in place.

For each hazard retained for further assessment, this review evaluated whether risk management was warranted for the importation of bovine in vitro produced embryos. If it was concluded that risk management was warranted, then the biosecurity measures were reviewed to determine if they were appropriate. If it was concluded that those biosecurity measures were not appropriate to achieve Australia's ALOP, alternative and/or complementary biosecurity measures were recommended.

- 4) Monitoring and review—the ongoing process by which the risk management measures are continuously audited to ensure that they are achieving the results intended.

The Department of Agriculture and Water Resources is responsible for implementing, monitoring and reviewing biosecurity measures to enable the safe importation of commodities into Australia, including bovine germplasm. The biosecurity measures were reviewed in the context of updated scientific information, including expert advice where available, as well as operational practicality. Stakeholder feedback received in the consultation phase of this review provided guidance to identify issues of concern relevant to the importation of frozen bovine in vitro produced embryos from Canada and the United States.

2.6 Risk communication

Risk communication is defined by the OIE Code as:

The process by which information and opinions regarding hazards and risks are gathered from potentially affected and interested parties during a risk analysis, and by which the results of the risk assessment and proposed risk management measures are communicated to the decision-makers and interested parties in the importing and exporting countries. It is a multidimensional and iterative process and should ideally begin at the start of the risk analysis process and continue throughout. (OIE 2016f)

In conducting BIRAs and reviews of risk management components, the Department of Agriculture and Water Resources consults directly with the Australian Government Department of Health to ensure that public health considerations are included in the development of Australia's animal biosecurity requirements. Furthermore, a formal process of consultation with external stakeholders is a standard procedure for all BIRAs and reviews to enable stakeholder assessment and feedback on the conclusions and recommendations about Australia's animal biosecurity policies.

3 Review of hazard identification

Hazard identification is the first component of the risk review process. It is described in the OIE Code (Article 2.1.2.) as a classification step that is undertaken to identify potential hazards that may be associated with the importation of a commodity, in this case, the importation of frozen bovine in vitro produced embryos (OIE 2016f).

3.1 Hazard identification

The list of diseases (hazards) of potential biosecurity concern was compiled from:

- diseases listed by the OIE as affecting cattle (OIE 2016d)
- diseases identified in the current import conditions for bovine semen and in vivo derived embryos
- other diseases identified as occurring in cattle.

The method of hazard identification and refinement is described in Section 2.3. The preliminary list of diseases/disease agents is shown in Table 3.

Table 3 summarises the results of the hazard refinement process, including the reason for removal or retention of each identified hazard.

Due to their largely ubiquitous occurrence and the numerous species, external parasites (e.g. ticks, fleas, mites) and internal parasites (e.g. helminths, nematodes) were not specifically included in the hazard identification list (Table 3), with the exception of parasitic diseases that are either OIE-listed or were considered in the context of emerging threats to biosecurity.

Many disease agents of potential biosecurity concern associated with the importation of in vitro produced embryos are opportunistic or ubiquitous, and/or the relevance of cattle in disease epidemiology is uncertain due to limited or insufficient information. It was appropriate to list these disease agents here, not only to indicate that they were considered, but also in the event that significant evidence of the role of frozen bovine in vitro produced embryos in disease spread is identified following completion of this review. These agents include:

Viruses

Schmallenberg virus, bluetongue virus, bovine herpes virus 4, bovine immunodeficiency virus, bovine viral diarrhoea virus, Crimean Congo haemorrhagic fever virus, bovine enterovirus, bovine leukemia virus, epizootic haemorrhagic disease virus, foot and mouth disease virus, bovine herpesvirus type 1, lumpy skin disease virus, bovine parainfluenza virus type 3, rabies virus, Rift Valley fever virus, rinderpest virus, vesicular stomatitis virus.

Bacteria

Anthraxis bacillus, *Brucella* spp. – *B. abortus*, *B. melitensis*, *B. suis*, *Mycobacterium avium* subsp. *paratuberculosis*, *Coxiella burnettii*, *Ehrlichia ruminantium*, *Campylobacter fetus* subsp. *venerealis*, *Mycobacterium bovis*, *Chlamydia* spp. – *C. psittaci*, *C. abortus*, *C. pecorum*, *Mycoplasma mycoides* subsp. *mycoides* SC, *Escherichia coli* 09:K99, *Histophilus somnus*, *Pasteurella multocida* – serotypes B2 and E2, *Leptospira hardjo-bovis*.

Protozoa

Anaplasma marginale, *Babesia* spp. – *B. bovis*, *B. bigemina*, *Theileria* spp. – *T. parva*, *T. annulata*, *Tritrichomonas foetus*, *Trypanosoma* spp. – *T. evansi*, *T. congolense*, *T. brucei*, *T. vivax*.

Prions

Bovine spongiform encephalopathy prions.

Table 3 Hazard identification and refinement – bovine in vitro produced embryos from Canada and US

Disease (Disease agent)	Susceptible species	OIE listed	Disease status – Notifiable			Country Status ⁽¹⁾ – Present			Risk status in bovine in vitro produced embryos		Retained for review Yes / No ⁽²⁾
			Australia	United States	Canada	Australia	United States	Canada	Evidence of transmission via bovine germplasm	OIE Code (Article)	
VIRAL DISEASES											
Infection due Schmallenberg virus (SBV) and viruses of Simbu serogroup (Schmallenberg virus and other Simbu viruses)	Cattle Also sheep, goats	No	No	No	No	Schmallenberg disease not reported Akabane, Aino viruses reported	Not reported	Not reported	Evidence of SBV antigen in bovine semen	No SBV in OIE Technical Factsheet	Yes
Bluetongue (Bluetongue virus)	Cattle, bison, buffalo Also sheep, goats	Yes	Clinical disease only	Notifiable in many States	Yes	Present– limited to zones	Present	Present– limited to zones	Evidence of virus in bovine semen and BTV-8 in in vivo derived embryos	Yes 8.3.11 8.3.12	Yes
Infection due to bovine herpesvirus 4 (Bovine herpesvirus 4)	Cattle Also buffalo, bison, sheep, goats	No	No	No	No	Not reported	Present	Present	Evidence of virus in bovine semen and in vivo derived embryos	No In OIE Code for bovine in vivo derived embryo	Yes
Infection due to bovine immuno- deficiency virus (Bovine immuno- deficiency virus)	Cattle	No	No	No	No	Present	Present	Present	Evidence in leucocyte fraction in semen	No In OIE Code for bovine in vivo derived embryo	No

Disease (Disease agent)	Susceptible species	OIE listed	Disease status – Notifiable			Country Status ⁽¹⁾ – Present			Risk status in bovine in vitro produced embryos		Retained for review Yes / No ⁽²⁾
			Australia	United States	Canada	Australia	United States	Canada	Evidence of transmission via bovine germplasm	OIE Code (Article)	
Bovine viral diarrhoea (Bovine pestivirus)	Cattle Also sheep, pigs	Yes Both types	Type 2	No	No	Type 2 absent	Present	Present	Evidence of virus in bovine semen and embryos	No In OIE Code for bovine in vivo derived embryos and semen	Yes
Crimean Congo haemorrhagic fever (Crimean Congo haemorrhagic fever virus)	Cattle Also sheep, goats, hares, dogs, mice, humans	Yes	Yes	Yes	No	Free	Free	Never reported	No evidence of sexual transmission in animals	No	No
Infection due to enterovirus (Bovine enterovirus)	Cattle	No	No	No	No	Present	Present	Present	Evidence of virus in bovine semen	No In OIE Code for bovine in vivo derived embryos	No
Enzootic bovine leucosis (Bovine leukemia virus)	Cattle Also sheep	Yes	Yes	No	No	Free only in dairy	Present	Present	Evidence in leucocyte fraction in semen	Yes 11.8.7	Yes
Epizootic haemorrhagic disease (Epizootic haemorrhagic disease virus)	Cattle Also deer	Yes	Yes	No	Yes	Virus present (No clinical disease)	Disease limited to zones.	Clinical disease in wildlife	Not known	Yes 8.7.11 8.7.12	Yes

Disease (Disease agent)	Susceptible species	OIE listed	Disease status – Notifiable			Country Status ⁽¹⁾ – Present			Risk status in bovine in vitro produced embryos		Retained for review Yes / No ⁽²⁾
			Australia	United States	Canada	Australia	United States	Canada	Evidence of transmission via bovine germplasm	OIE Code (Article)	
Foot and mouth disease (Foot and mouth disease virus)	Cloven hoofed animals	Yes	Yes	Yes	Yes	Free without vaccination	Free without vaccination	Free without vaccination	Evidence of virus in bovine semen	Yes 8.8.18 8.8.19	Yes
Infectious bovine rhinotracheitis/ Infectious pustular vulvovaginitis (Bovine herpesvirus-1)	Cattle	Yes	No Notifiable in Victoria	No	No	1.2b present 1.2a & 1.1 absent	Present	Present	Evidence of virus in bovine semen and embryos not washed in trypsin	Yes 11.10.8	Yes
Lumpy skin disease (Lumpy skin disease virus)	Cattle Also buffalo	Yes	Yes	Yes	Yes	Free	Free	Free	Evidence of virus in bovine semen	Yes 11.11.9 11.11.10	Yes
Infection due to parainfluenza-3 virus (Parainfluenza -3 virus)	Cattle	No	No	No	No	Present	Present	Present	Not known. Virus is widespread and has no known economic significance	No In OIE Code for bovine in vivo derived embryos	No
Rabies (Rabies virus)	Cattle Other warm blooded mammals	Yes	Yes	Yes	Yes	Free	Present	Present	No evidence in bovine germplasm	No	No
Rift Valley fever (Rift Valley fever virus)	Cattle, buffalo Also sheep, goats, camelids	Yes	Yes	Yes	Yes	Free	Free	Free	No clear evidence of transmission	Yes 8.14.10	Yes

Disease (Disease agent)	Susceptible species	OIE listed	Disease status – Notifiable			Country Status ⁽¹⁾ – Present			Risk status in bovine in vitro produced embryos		Retained for review Yes / No ⁽²⁾
			Australia	United States	Canada	Australia	United States	Canada	Evidence of transmission via bovine germplasm	OIE Code (Article)	
Rinderpest (Rinderpest virus)	Cattle Also buffalo	Yes	Yes	Yes	Yes	Free	Free	Free	No clear evidence of transmission	NA	No Now globally eradicated
Vesicular stomatitis (Vesicular stomatitis virus)	Cattle, horses, pigs and humans	No	Yes	Yes	Yes	Free	Present	Reported in 1949	No clear evidence of transmission	No In OIE Code for bovine in vivo derived embryos	Yes
BACTERIAL DISEASES											
Anthrax (<i>Anthraxis bacillus</i>)	Mammals	Yes	Yes.	Yes	Yes	Present– limited distribution	Present– limited to zones	Present– known in wild animals	No evidence in bovine germplasm	No	No
Brucellosis due to i. <i>Brucella abortus</i>	Cattle, humans, sheep, goats, camels, dogs	Yes	Yes	Yes	Yes	Free	Present– limited to zones	Present in wildlife– limited to zones. Last case in 1989	Present in semen but evidence of transmission not clear	Yes 8.4.18	Yes
ii. <i>B. melitensis</i>	Cattle, humans, sheep, goats, camels, dogs		Yes	Yes	Yes	Free	Free	Free	No evidence in bovine germplasm		
iii. <i>B. suis</i>	Cattle, humans, pigs, dogs		Yes	Yes	Yes	Present	Present	Present – not porcine	No evidence in bovine germplasm		

Disease (Disease agent)	Susceptible species	OIE listed	Disease status – Notifiable			Country Status ⁽¹⁾ – Present			Risk status in bovine in vitro produced embryos		Retained for review Yes / No ⁽²⁾
			Australia	United States	Canada	Australia	United States	Canada	Evidence of transmission via bovine germplasm	OIE Code (Article)	
Paratuberculosis (<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>)	Cattle, cattle strain may infect other ruminants	Yes	Yes	No	No	Present. Industry control and management programmes in place	Present	Present	Present in germplasm but evidence of transmission not clear	No In OIE Code for bovine in vivo derived embryos	Yes
Q fever (<i>Coxiella burnettii</i>)	Cattle, Multiple other species inc humans	Yes	No	No	No	Present	Present	Present	Present in semen but evidence of transmission not clear	No In OIE Code for bovine in vivo derived embryos	No
Heartwater (<i>Ehrlichia ruminantium</i>)	Cattle Multiple other species	Yes	Yes	Yes	Yes	Free	Free	Free	No clear evidence of transmission	No	No
Bovine genital campylobacteriosis (<i>Campylobacter fetus</i> subsp. <i>venerealis</i>)	Cattle	Yes	No	No	No	Present	Present	Present	Evidence of transmission via bovine semen	No In OIE Code for bovine semen	Yes
Bovine tuberculosis (<i>Mycobacterium bovis</i>)	Cattle, bison, buffalo Multiple other species, humans	Yes	Yes	Yes	Yes	Free	Present– limited to one or more zones	Present (Last In 2011)	Present in semen but evidence of transmission not clear	Yes 11.5.8	Yes

Disease (Disease agent)	Susceptible species	OIE listed	Disease status – Notifiable			Country Status ⁽¹⁾ – Present			Risk status in bovine in vitro produced embryos		Retained for review Yes / No ⁽²⁾
			Australia	United States	Canada	Australia	United States	Canada	Evidence of transmission via bovine germplasm	OIE Code (Article)	
Chlamydiosis <i>Chlamydia psittaci</i> , <i>C. abortus</i> , <i>C. pecorum</i>	Cattle	No	No	No	No	Present (Free of <i>C.</i> <i>abortus</i>)	Present	Present	Present in semen but evidence of transmission not clear	No In OIE Code for bovine in vivo derived embryos	No
Contagious bovine pleuropneumonia (<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> SC)	Cattle	Yes	Yes	Yes	Yes	Free	Free (Last in 1892)	Free (Last in 1876)	Present in semen but evidence of transmission not clear	Yes 11.7.12	Yes
Coliform infection due to <i>E. coli</i> <i>Escherichia coli</i> 09:K99	Cattle, sheep, pigs	No	No	No	No	Present	Present	Present	Strains of <i>E. Coli</i> are common contaminants of semen	No In OIE Code for bovine in vivo derived embryos	No
Histophilosis <i>Histophilus somni</i>	Cattle, bison, sheep	No	No	No	No	Present	Present	Present	Present in genital tracts and semen and transmission via semen is suspected	No	No
Haemorrhagic septicaemia (<i>Pasteurella multocida</i> – strains 6b and 6e)	Cattle Also buffaloes, sheep, goats, pigs	Yes	Yes	Yes	Yes	Free	Present (1969 last occurrence)	Free	Not known	No	No

Disease (Disease agent)	Susceptible species	OIE listed	Disease status – Notifiable			Country Status ⁽¹⁾ – Present			Risk status in bovine in vitro produced embryos		Retained for review Yes / No ⁽²⁾
			Australia	United States	Canada	Australia	United States	Canada	Evidence of transmission via bovine germplasm	OIE Code (Article)	
Leptospirosis (<i>Leptospira hardjo-bovis</i>)	All vertebrates except birds	No	No	No	No	Present	Present	Present	Present in germplasm. Evidence of transmission not clear	No In OIE Code for bovine in vivo derived embryos	Yes
PARASITIC DISEASES											
Bovine anaplasmosis (<i>Anaplasma marginale</i>)	Cattle	Yes	Yes – in tick free areas only	No	Yes	Present– limited to zones	Present	Present– limited to zones	Not known	No In OIE Code for bovine in vivo derived embryos	Yes
Bovine babesiosis (<i>Babesia bovis</i> , <i>B. bigemina</i>)	Cattle Also buffalo	Yes	Yes	Yes	Yes	Present– limited to zones	Last reported 1943	Free	No evidence in bovine germplasm	No	No
Theileriosis (<i>Theileria.parva</i> , <i>T. annulata</i>)	Cattle	Yes	Yes	Yes	Yes	Free	Free	Free	No evidence in bovine germplasm	No	No
Trichomonosis (<i>Tritrichomonas foetus</i>)	Cattle	Yes	No	No	No	Present	Present	Present	Evidence of transmission via bovine semen	No In OIE Code for bovine semen	Yes

Disease (Disease agent)	Susceptible species	OIE listed	Disease status – Notifiable			Country Status ⁽¹⁾ – Present			Risk status in bovine in vitro produced embryos		Retained for review Yes / No ⁽²⁾
			Australia	United States	Canada	Australia	United States	Canada	Evidence of transmission via bovine germplasm	OIE Code (Article)	
Trypanosomoses (<i>Trypanosoma</i> spp. – <i>T. evansi</i> , <i>T. congolense</i> , <i>T. brucei</i> , <i>T. vivax</i>)	Cattle Also multiple other species	Yes	Yes	Yes	Yes	Free	Free	Free	Not known	No	No
PRION DISEASES											
Bovine spongiform encephalopathy	Cattle	Yes	Yes (TSE)	Yes	Yes	Free OIE negligible risk status	Last in 2012 OIE negligible risk status	OIE controlled risk status (Last case in 2015)	No evidence in bovine germplasm	No In OIE Code for bovine in vivo derived embryos	Yes

NOTES:

NA – Not available.

(1) The terminology used for country status is based on OIE World Animal Health Information System (WAHIS) categorisation.

(2) Where Canada and the United States are free from certain diseases, country freedom certification can be provided by the relevant competent authority, to manage biosecurity risks from importation of bovine in vitro produced embryos to Australia.

Sources: OIE 2016f, OIE 2016d.

4 Disease risk review

4.1 Schmallerberg and other viruses of Simbu serogroup

4.1.1 Background

There are a number of arboviruses belonging to the Simbu serogroup in the family Bunyaviridae (Nichol et al. 2005). The most notable of those that infect livestock and which cause embryonic and foetal losses and multiple congenital deformities in cattle are Schmallerberg, Akabane and Aino viruses. Schmallerberg virus causes reproductive losses in sheep, goats and deer while Akabane virus causes reproductive losses in sheep (Beer, Conraths & van der Poel 2013). Schmallerberg virus (SBV) is reported only in Europe (Beer, Conraths & van der Poel 2013), though cattle seropositive to SBV were reported in Tanzania and Mozambique (Blomström et al. 2014; Mathew et al. 2015). Akabane virus is reported throughout parts of Asia, Africa, the Middle East and Australia but is not present in North or South America and Europe. Aino virus is reported in parts of Australia, Japan and Korea.

Phylogenetic analysis indicate that SBV belongs to the Sathuperi virus group (Goller et al. 2012). Recent genetic analyses of Akabane virus isolates have described four genetically discrete groups (I–IV) and one subgroup (Ia–Ib) (An et al. 2010). Group I contains strains from Japan, Group II contains strains from Japan, Taiwan and Israel. Australian strains belong to group III and Kenyan strains belong to group IV. Strains vary in virulence, affecting the proportion of foetuses which become infected in utero (St George et al. 2001). A highly virulent Asian strain, the Iriki strain which belongs to Group Ia, can cause encephalitis in mature cattle in Asia (Brenner 2007; Lee et al. 2002; Liao et al. 1996; Miyazato et al. 1989).

Antibodies to SBV were detected in both domestic ruminants (cattle, sheep, goats and camelids) and wild ruminants (buffalo, bison and deer) (EFSA, 2014). Antibodies to Akabane virus have been detected in cattle, goats, sheep, buffalo, deer, camels, horses, dogs and African wildlife species (OIE 2016c). In Australia, Akabane antibodies have not been found in pigs, marsupials or humans (St George et al. 2001). Antibodies to Aino virus are limited mainly to cattle, buffalo and sheep (Cybinski & St George 1978).

SBV is not reported in Australia. Akabane disease and the disease caused by the closely related Aino virus are endemic in parts of northern and eastern Australia. Schmallerberg, Akabane and Aino disease are not nationally notifiable. The distribution of Akabane virus and its vectors in Australia is monitored by the National Arbovirus Monitoring Program (AHA 2015b). The distribution of Akabane and Aino is limited by the distribution of competent culicoides vectors. There are large areas in Australia that are free from these viruses because vectors are absent or in low numbers.

Akabane disease and infection due to Aino virus and SBV are not OIE-listed diseases (OIE 2016d) but they are significant for international trade because some countries, which claim freedom from these diseases, require animal health certification for Schmallerberg, Akabane and/or Aino virus infection for trade in live sheep, goats, cattle and genetic material.

4.1.2 Technical information

Pathogenesis

Cattle are infected by the bites of infected insects and the animal becomes viraemic within 3–4 days. Viraemia may last 1–9 days, most commonly 2–6 days (Beer, Conraths & van der Poel 2013; Radostits et al. 2007). This is followed by a humoral response which rapidly clears virus from the circulation (St George et al. 2001). In endemic areas, antibody in female animals prevents foetal infection. However, Schmallerberg, Akabane and/or Aino virus infection of susceptible cows at 30–150 days gestation results in virus crossing the placenta and viral multiplication in rapidly dividing foetal cells (OIE 2016c; St George et al. 2001). Depending on the stage of gestation, the virus causes non-inflammatory necrosis in brain, spinal cord and muscle cells in the developing foetus (OIE 2016c).

Clinical signs

Schmallerberg, Akabane and Aino virus infection in adult cattle are usually subclinical, although abortion and dystocia may occur in cows carrying an affected foetus. Encephalomyelitis is a rare occurrence with Akabane virus infection in adult cattle (Lee et al. 2002).

Foetal abnormalities observed depend on age of foetus when infection occurs, with hydrancephaly at 76–104 days and arthrogryposis at 103–174 days gestation in cattle (Kirkland et al. 1988). Thus reproductive losses in herds with long calving seasons often begin with stillbirths and abortions, followed by the birth of uncoordinated calves, then calves with arthrogryposis and muscle changes, and lastly hydrancephaly and other severe central nervous system lesions (OIE 2016c).

Epidemiology

Schmallerberg, Akabane and Aino viruses are non-contagious, being biologically transmitted by biting midges, particularly *Culicoides* spp. and sometimes mosquitoes (Bishop et al. 1996; OIE 2016c; St George et al. 2001). Schmallerberg virus first emerged in Europe in November 2011 and had spread across Europe by the end of 2012 (Beer, Conraths & van der Poel 2013).

Transmission requires a population of competent adult vectors, favourable climatic conditions for virus amplification in the vectors and sufficient viral load to initiate infection and amplification in cattle. Outbreaks of disease occur when susceptible cattle in early pregnancy are exposed to an increase in vector populations, especially if the virus has been absent from the area for several years and herd immunity has waned (OIE 2016c).

Diagnosis

Schmallerberg, Akabane and Aino disease is usually diagnosed by antibody testing of foetal fluids or precolostral serum from calves or lambs (haemagglutination inhibition, complement fixation, serum neutralisation tests and competitive enzyme-linked immunosorbent assays) and histopathology on foetal or neonatal brain, spinal cord and muscle. Virus can be isolated from the blood of viraemic animals and occasionally from foetal material (St George et al. 2001) using mammalian and insect cell culture as well as yolk sac inoculation and intracerebral inoculation of suckling mice. Virus or antigen is identified by fluorescent antibody, immunohistochemistry or neutralisation tests and PCR techniques (Kirkland 2002; OIE 2016c).

4.1.3 Transmission in germplasm

Semen

Evidence for excretion of live infective virus in semen is not conclusive.

SBV antigen can be excreted in bull semen and experimental transmission to mice has resulted in antibodies to SBV (Ponsart et al. 2014; Schulz et al. 2015).

Akabane virus was not excreted in the semen of eight artificially infected bulls (Parsonson et al. 1981) but two of 16 sheep inoculated with semen from naturally infected bulls developed antibody to Akabane virus, thus suggesting virus in the bulls' semen (Gard, Melville & Shorthose 1989). As published research or reports on Akabane or Aino viruses in the semen of donors are not available, it is not known if these viruses can be excreted in semen.

These results indicate SBV, Aino and Akabane viral antigen can be excreted in semen and can cause antibody production in sheep and mice. However, it is not known if the viruses in the semen can infect recipient cows or heifers.

Embryos

There is no information regarding infection of in vitro produced embryos with SBV.

Published research or reports on venereal transmission (that is, in vivo transmission) of Akabane or Aino viruses are not available. Singh et al. (1982a) had conducted a study where in vivo derived embryos were exposed in vitro to Akabane virus and washed according to procedures described in the IETS Manual (IETS 2010). The virus was not isolated from the washed embryos.

Consequently, the OIE Code Article 4.7.14 ranks Akabane in cattle as an IETS Category 4 disease, that is, a disease for which studies have been done, or are in progress, that indicate that no conclusions are yet possible with regard to the level of transmission risk; or the risk of transmission via in vivo derived embryo transfer might not be negligible even if the embryos are properly handled according to the IETS Manual between collection and transfer (OIE 2016f).

4.1.4 Current biosecurity measures

Australia has animal biosecurity measures for Schmallenberg virus for bovine semen and in vivo derived embryos from Canada and the United States.

4.1.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by Schmallenberg, Akabane and Aino viruses:

- Schmallenberg, Akabane and Aino viruses cause embryonic and foetal losses and multiple congenital deformities in cattle. Schmallenberg virus also cause reproductive losses in sheep, goats and deer. Akabane (but not Aino viruses) also cause reproductive losses in sheep.
- Antibodies to Schmallenberg and Akabane virus have been detected in cattle, goats, sheep, buffalo, and deer. Akabane virus antibody was also detected in camels, horses, dogs and African wildlife species. Antibodies to Aino virus are limited mainly to cattle, buffalo and sheep.

- Schmallerberg, Akabane and Aino virus infection in adult animals is usually subclinical, although abortion and dystocia may occur in cows carrying an affected foetus.
- Infection due to Schmallerberg, Akabane and Aino viruses are not OIE-listed diseases or nationally notifiable in Australia.
- Schmallerberg virus is not reported in Australia, Canada and the United States. Akabane and Aino viruses are present across northern Australia but Akabane and Aino viruses are not reported in Canada and the United States.
- Schmallerberg and Akabane virus antigen have been detected in bovine semen.
- Given the presence of competent *Culicoides* spp. in parts of Australia, there is a risk of entry, establishment and spread of SBV. This may result in significant reproductive losses in cattle, sheep, goats and deer. The current distribution of competent vectors in Australia is likely to result in a viral distribution similar to that currently seen for Akabane and Aino viruses.

4.1.6 Conclusion

Based on the preceding factors, it was concluded that there is a risk of transmission of Schmallerberg viruses via bovine in vitro produced embryos. Akabane and Aino viruses are present in Australia. None are present in Canada or the United States. Therefore animal biosecurity measures for bovine in vitro produced embryos will be required for only SBV.

The following biosecurity measures provide appropriate risk management.

For bovine in vitro produced embryos from Canada and the United States:

- No cases of disease caused by Schmallerberg viruses have been detected or reported in Canada/the United States.

4.2 Bluetongue

4.2.1 Background

Bluetongue (BT) is an insect-borne viral disease of ruminants, characterised in sheep by cyanosis of mucous membranes with widespread haemorrhages and oedema.

BT is enzootic in most countries between 53° North and 34° South with occasional epizootics outside these latitudes. There are 26 serotypes of BT virus (BTV) distributed worldwide that may be differentiated on the basis of topotype or nucleotype. Viruses of identical serotypes have been isolated in widely different geographical areas. The different wild type strains of BTV vary in virulence and laboratory strains can differ in virulence, pathogenesis and epidemiology from related wild type strains.

Twelve BTV serotypes (1, 2, 3, 5, 7, 9, 12, 15, 16, 20, 21 and 23) have been identified in Australia from insects or clinically healthy sentinel cattle but clinical disease has not been seen in cattle or reported in commercial sheep flocks or goats in Australia (AHA 2015b; Geering, Forman & Nunn 1995a).

Serotype classification is related to the genes that determine specific surface antigens. While vaccine is protective for all identical serotypes the genetic makeup of these viruses can be quite different in areas of the genome that influence virulence. Serotypes can therefore not be directly compared without genome sequencing.

All ruminant species are susceptible. Of the domestic species, sheep and white-tailed deer are the most severely affected, while goats are occasionally clinically affected. Cattle are generally subclinically infected but are the major vertebrate amplifier of the virus. The clinical picture is very much dependent on the viral genome.

BT is regularly reported in the United States (serotypes 1, 2, 3, 5, 6, 9, 10, 11, 12, 13, 14, 17, 19, 22 and 24) (MacLachlan et al. 2013). The United States once conducted regular BTV surveillance to determine which States could be classed as bluetongue low incidence States but this now does not appear to be the case.

BT is occasionally reported in Canada, usually due to BTV-1 within the Okanagan Valley in British Columbia (Paré et al. 2012). Although there are now no semen collection centres in the Okanagan Valley, cattle production is a significant industry there. In August 2015, Canada reported BTV-13 in southwest Ontario, the first time the virus has been found in livestock on a birth farm outside the Okanagan (OIE 2016e).

BT is an OIE-listed disease (OIE 2016d). In Australia, the distribution of BTV and its vectors in Australia is monitored by the National Arbovirus Monitoring Program (AHA 2015b). The distribution of BTV is limited by the distribution of competent culicoides vectors. There are large areas in Australia that are free from BTV because vectors are absent or in low numbers. Clinical BT is nationally notifiable and is classified as an EADRA Category 3 disease. An EADRA category 3 disease is a disease that has the potential to cause significant national socio-economic consequences through its impact on international trade, market disruptions involving two or more states and severe production losses to affected industries. Category 3 diseases have minimal or no effect on human health or the environment (AHA 2010; Department of Agriculture and Water Resources 2016).

4.2.2 Technical information

Pathogenesis

Viraemia is detectable 2–3 days post-infection and often lasts less than four weeks but can in exceptional cases persist for eight weeks (Bonneau et al. 2002; Gard 1998; Gard & Melville 1992; Koumbati et al. 1999; Melville et al. 2005b; Richards et al. 1988; Singer, MacLachlan & Carpenter 2001). The OIE Code (OIE 2016f) defines the infective period for BTV as 60 days (Article 8.3.1).

The pathogenesis of BTV is similar in all species of ruminants but susceptibility to clinical disease varies markedly between species and viral genotypes. After infection of the skin through the bite of an infected vector, the virus replicates in a regional lymph node. BTV is highly cell-associated, particularly in blood cells and endothelial cells, and disseminates to a variety of tissues (MacLachlan & Gard 2009). BTV has been isolated from most tissues including the spleen, thymus, lungs, liver, kidney, prescapular, prefemoral and mesenteric lymph nodes, bone marrow and trapezius muscle of experimentally inoculated calves (Barratt-Boyes & MacLachlan 1994; Barratt-Boyes & MacLachlan 1995; MacLachlan et al. 1990). Experimental infection of sheep with the European strain of BTV-8 resulted in detection of BTV RNA in spleen; lung; tonsils; prescapular, mesenteric, popliteal and iliac lymph nodes; liver; uterus; kidney; Peyer's patches; myocardial and skeletal muscle; mammary gland; brain; adrenal gland; abomasum and liver (Worwa et al. 2010). BTV is found transiently in serum and monocytes but is present within red blood cells, even in the presence of high antibody titres, for up to eight weeks (Schwartz-Cornil et al. 2008). Infection with one serotype does not confer immunity to other serotypes.

In animals that show clinical signs, BTV causes injury to blood vessel walls that results in oedema, hyperaemia, haemorrhages and infarction, especially of the respiratory and upper gastrointestinal tracts. Disseminated intravascular coagulation may also occur in severely affected sheep and white-tailed deer (MacLachlan & Gard 2009).

Clinical signs

In cattle, infection is usually subclinical. However, clinical signs in cattle have been reported with some serotypes, particularly the European BTV-8 (European Food Safety Authority 2008). The incubation period is four to eight days. Viraemia is detectable three days post infection, and often lasts less than four weeks, but may in exceptional cases persist for eight weeks. Antibodies are usually first detected around one to two weeks post infection and remain detectable for at least 60 days. Reports of carrier states are generally confined to older literature before researchers recognised the significance of multiple re-infection of animals with different serotypes of BTV (Geering, Forman & Nunn 1995a).

In sheep, infection with some serotypes causes fever, hyperaemia and cyanotic oedema of buccal and nasal mucosa, membranes, skin and coronary bands, muscle degeneration and/or foetal infection with abortion and congenital abnormalities. The incubation period generally ranges from 4 to 7 days and viraemia lasts about seven days, with a maximum of 20 days. In goats, the clinical signs are usually less severe than those in sheep.

Epidemiology

The distribution of BTV depends on presence and density of animal reservoirs, amplifying hosts such as free living ruminants and cattle, a population of competent adult *Culicoides* vectors,

favourable climatic conditions for virus amplification in the vectors and sufficient viral load to initiate infection and amplification in cattle.

BTV was generally regarded as non-contagious, being biologically transmitted by *Culicoides* spp. until transplacental transmission of the northern European strain of BTV-8 was demonstrated (De Clercq et al. 2008). Although there are over 1400 species of *Culicoides* worldwide, less than 20 are known vectors of BTV.

The OIE Code provides guidelines for BTV surveillance of domestic animals and vectors and standards for establishing country or zone freedom from disease. Countries between latitudes 53° North and 34° South require an ongoing surveillance system that demonstrates no evidence of either BTV transmission or competent vectors for the previous two years in order to be recognised as a country or zone free from BTV (OIE 2016f). Thus any country within these latitudes not having such a surveillance system in place can be recognised as infected.

Morbidity can be up to 100%. Mortality in sheep and goats usually ranges from 0 to 20% but may be up to 70% in highly susceptible sheep breeds. Mortality is very rare in cattle, from 0.01% for most serotypes to 3% for the European BTV-8 showing some clinical signs.

In infected herds, most animals already have antibodies against at least one serotype with immunity to that serotype. However, they would still be susceptible to infection by other serotypes. Susceptible animals are most likely to become infected during the late summer and early autumn and remain infectious for up to eight weeks.

Live attenuated and inactivated vaccines are available to limit livestock losses and reduce circulation of BTV. Live attenuated vaccines can provide immunity lasting at least a year against relevant serotypes, but may have adverse consequences, including teratogenicity during early pregnancy, and ongoing spread by vectors with possible re-assortment of vaccine virus genes with those of wild type virus. Inactivated vaccines require a second shot to boost the antibody titre.

Diagnosis

The diagnostic tests for BTV, prescribed for international trade in the OIE Manual (OIE 2016c), are antibody detection by competitive enzyme linked immunosorbent assay (cELISA) of blood or bulk milk samples, and agent identification by viral isolation or reverse transcriptase-polymerase chain reaction (RT-PCR) assay. Serological responses appear some 7–14 days after BTV infection and are generally long-lasting. Antibody to Australian BTV serotypes was reliably detected in naturally infected cattle for four years by cELISA (Melville et al. 2005a).

Both the cELISA and OIE nested RT-PCR or equivalent, provided they are regularly assessed with regular proficiency tests, are highly sensitive and specific tests for BTV and are effective in determining donor BTV status (Batten et al. 2008a; Batten et al. 2008b).

4.2.3 Transmission in germplasm

Semen

Evidence of pathology caused by infection with BTV in the male genital tract is equivocal. BTV has been reported to produce focal degeneration of seminiferous tubules with spermatozoid abnormalities in bulls (Foster et al. 1980), yet it has also been reported as not causing gross

pathological changes to the genital tract of bulls (Grocock, Parsonson & Campbell 1983; Parsonson et al. 1987).

In Australia, mature bulls experimentally infected with laboratory adapted BTV or with wild-type unadapted serotype 23, recognised to be the most virulent serotype in Australia, can excrete the virus in semen during early viraemia, even in the presence of circulating antibody. Virus may be detected in semen for up to ten days after viraemia has passed but the virus could not be identified in the semen of young bulls experimentally infected with either laboratory cultured or wild-type unadapted virus (Melville & Kirkland 1994).

There is no evidence of a significant difference in the viraemic period of different serotypes (EFSA 2007; EFSA 2008). BTV-8 has characteristics in common with laboratory-adapted strains, and can be shed in semen (Vanbinst et al. 2010). The time of year that semen is collected may modify the risk of its contamination with BTV. Semen that is collected during the period of low vector activity, when BTV transmission is reduced, is less likely to be infected with virus.

Embryo

Early studies in cattle showed that despite adherence of BTV to bovine embryos compared with other viruses (Gillespie et al. 1990), IETS washing and handling procedures appeared to consistently remove the risk of infecting recipients with both laboratory-adapted and field strains of virus (Acree et al. 1991; Bowen et al. 1983a; 1983b; Singh et al. 1982a; Thomas, Singh & Hare 1983; 1985). However, following an outbreak of a new strain of BTV-8 in Europe in 2006, research showed that IETS washing and trypsin treatment did not succeed in removing BTV-8 from in vitro spiked in vivo derived bovine embryos. The transference of washed and treated embryos resulted in viraemia in the recipient cows (Vandaele et al. 2011).

Despite the findings with BTV-8, the OIE Code Article 4.7.14 categorises bluetongue in cattle as an IETS Category 1 disease, that is, a disease for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the in vivo derived bovine embryos are properly handled (that is, no embryos with defective zona pellucida) and washed between collection and transfer (OIE 2016f).

There is no evidence that IETS washing and trypsin treatment can remove BTV from bovine in vitro produced embryos. A recent study showed that IETS washing and trypsin treatment failed to remove BTV-8 from bovine in vitro produced embryos (Penido et al. 2013).

4.2.4 Current biosecurity measures

Australia has animal biosecurity measures for BTV for bovine semen but not for bovine in vivo derived embryos from Canada and the United States. The animal biosecurity measures require certification of semen donors in BTV free zones, negative antibody test or negative antigen test.

Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by BTV:

- BT is an OIE-listed disease and clinical bluetongue is nationally notifiable in Australia.
- BT is regularly reported in the United States and occasionally in Canada. There is now no national surveillance for BTV to enable the United States Department of Agriculture (USDA)

to classify the BTV low incidence States and no semen collection centres in the Okanagan Valley in British Columbia, Canada.

- An insect-borne viral disease, BTV activity increases when vector activity peaks during late summer and autumn.
- Although BTV in cattle generally cause subclinical infection, cattle are the major vertebrate amplifier of the virus for spread by vectors. All ruminant species are susceptible to infection by these vectors, with sheep and white-tailed deer being the most severely affected, and goats occasionally affected.
- Some serotypes and strains of serotypes of BTV can infect bovine semen and embryos. Except for BTV-8, the virus can be removed from the in vivo derived embryos by washing procedures as described in the IETS Manual.
- Diagnostic testing of semen, embryo and oocytes donors using properly validated cELISA and/or nested RT-PCR or equivalent provide adequate risk management against risk of entry, establishment and spread of BTV via germplasm in Australia.

Conclusion

Based on the preceding factors, it was concluded biosecurity measures are required for bovine in vitro produced embryos.

For bovine in vitro produced embryos from Canada and the United States:

- Blood samples drawn from each donor
 - were subjected to a cELISA test to detect antibodies to the BTV group between 28 and 60 days after each collection of oocytes with negative results
 - or
 - were subjected to an agent identification test on a blood sample taken on the day of collection with negative results.
- OR
- All donors were kept in a country free or seasonally free from BTV as recognised by Australia* at least 60 days prior to, and at the time of, collection of oocytes.

[*Australia recognises Canada as a country seasonally free from BTV without testing between 1 January and 15 May, except the Okanagan Valley of British Columbia]

4.3 Infection due to bovine herpesvirus 4

4.3.1 Background

Bovine herpesvirus 4 (BoHV-4) is normally regarded as relatively non-pathogenic virus, but it has been implicated in a number reproductive problems in cattle, including infertility, abortion, vulvo-vaginitis, post-partum metritis and orchitis. The virus has been implicated in other clinical conditions, including pneumonia, keratoconjunctivitis, encephalitis, mastitis and diarrhoea (Chastant-Maillard 2015).

BoHV-4 is a member of the Herpesviridae family that infects ruminants, in particular cattle, bison, African buffalo, sheep and goats and some non-ruminants, in particular, guinea pigs, cats and lion (Ackermann 2006; Chastant-Maillard 2015).

The virus is generally regarded as having a world-wide distribution but it has not been reported in Australia. It is present in Canada and the United States (Ali et al. 2012).

BoHV-4 is neither an OIE-listed disease nor nationally notifiable in Australia.

4.3.2 Technical information

Pathogenesis

BoHV-4 transmission is both vertical and horizontal and also indirectly by fomites. Primary multiplication is within epithelial cells in the mucosa and dissemination is via infected mononuclear blood cells. The main targets for viral replication are the lymphoid organs, the epithelial cells of the upper respiratory tract, and the urogenital and alimentary tracts (Egyed et al. 1996). The lymphoid organs and mononuclear blood cells are possible primary sites of viral latency (Dubuisson et al. 1989), and the nervous system is believed to be involved in the persistence of the virus (Costa et al. 2011). Shedding of the virus occurs between 1 and 12 days post infection in the nasal and conjunctival discharges.

The virus displays tropism for endometrial and endothelial cells (Lin et al. 1997), with infection of vascular endothelia being the site of viral invasion into various tissues and organs, uterus and mammary gland, spleen, bladder, central nervous system, nerve ganglia, liver, lung, nasal mucosa, lymph nodes, thymus, tonsils and small intestine (Chastant-Maillard 2015; Costa et al. 2011; Egyed et al. 1996). It is also able to cross the placental barrier and intensively replicating within the foetus, due to its affinity for dividing cells.

After primary infection, a latent infection is established within lymphocytes and macrophages.

Clinical signs

BoHV-4 has been implicated in a number reproductive problems in cattle, and it believed BoHV-4 becomes pathogenic in association with other pathogenic agents, as bacteria, fungi or other viruses were also identified in 75% of BoHV-4 cases (Fábián et al. 2008; Frazier et al. 2001).

Epidemiology

BoHV-4 has been implicated in respiratory, reproductive, gastro-intestinal, lactational and ocular diseases, exacerbating the clinical impact of uterine, vaginal and mammary BoHV-4 infection with concomitant bacterial infections; its seroprevalence being higher in diseased animals than healthy animals. Although bovine fetuses can be infected in utero by BoHV-4, they are born as seronegatives (Chastant-Maillard 2015).

A marked feature, like other herpesviruses, is that the virus can cause latent infection in cattle, particularly in the macrophages and occasionally in the trigeminal ganglia of naturally infected cattle. However, experimental reactivation of BoHV-4 did not cause clinical signs (Dubuisson et al. 1989). Co-infection of cattle with three distinct bovine herpesviruses, BoHV-1, BoHV-4 and BoHV-5, appears to be a naturally occurring phenomenon (Campos et al. 2014).

Treatment is by providing general health support and antibiotics against concomitant bacterial infections.

Diagnosis

BoHV-4 antibodies can be detected in serum samples by indirect ELISA. Peripheral blood mononuclear cells can be tested by PCR for virus (de Boer et al. 2014).

4.3.3 Transmission in germplasm

Semen

BoHV-4 has been identified in bovine semen of healthy bulls in an Argentinian Semen Collection Centre (Morán et al. 2013) but the importance of this has not been evaluated.

Embryo

It has been observed that enveloped viruses such as BoHV-4 can adhere firmly to the zona pellucida and that thorough washing could not remove the virus. However, exposure to the enzyme trypsin has resulted in the removal of BoHV-4 from bovine in vivo derived embryos (Stringfellow et al. 1990)

The OIE Code Article 4.7.14 ranks BoHV-4 as an IETS Category 4 disease, that is, a disease for which studies have been done, or are in progress, that indicate no conclusions are yet possible with regard to the level of transmission risk; or the risk of transmission via in vivo derived embryo transfer might not be negligible even if the embryos are properly handled according to the IETS Manual between collection and transfer (OIE 2016f).

BoHV-4 virions were isolated from bovine granulosa cells and oocytes in abattoir-derived ovaries being prepared for in vitro production of bovine embryos, indicating that BoHV-4 ovary infections could occur regularly (González Altamiranda et al. 2015). These authors concluded there is a risk of BoHV-4 transmission via embryo transfer. This is in contrast to Chastant-Maillard (2015) who reviewed BoHV-4 infection of the reproductive tract and concluded that BoHV-4 does not appear as a risk for both in vivo derived and in vitro produced embryo production as does BoHV-1.

4.3.4 Current biosecurity measures

Australia has no animal biosecurity measures for BoHV-4 for bovine semen and in vivo derived embryos from Canada and the United States.

4.3.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by BoHV-4:

- Infection due to BoHV-4 is not an OIE-listed disease and is not nationally notifiable in Australia.

- Co-infection with other pathogenic agents such as bacteria appears necessary for BoHV-4 to have any clinical impact. As a hazard on its own, it is regarded as non-pathogenic.
- BoHV-4 has not been reported in Australia but is present in Canada and the United States.
- BoHV-4 can be present in bovine semen and embryos, but the risk of transmission via germplasm has not been established.

4.3.6 Conclusion

Based on the preceding factors, it is concluded that animal biosecurity measures are not warranted for BoHV-4 for bovine in vitro produced embryos from Canada and the United States.

4.4 Bovine viral diarrhoea

4.4.1 Background

Bovine pestivirus, also known as bovine viral diarrhoea virus (BVDV), is the cause of bovine viral diarrhoea (BVD) and mucosal disease (MD).

BVDV isolates are classified as non-cytopathic (NCP) or cytopathic (CP) biotypes. Infection of foetuses with the NCP biotype results in abortion or persistent infection (PI) with immunotolerance throughout its postnatal life. Superinfection with the homologous CP biotype in animals with PI causes MD. Most PI calves are born small and die from MD at a relatively young age. Some may show no clinical signs, becoming a major source of infection in a herd. Clinical infection with the NCP biotype in non-persistently infected animals results in BVD and viraemia is usually characterised by diarrhoea, its severity largely dependent on the pathogenicity of the virus.

Two genotypes, BVDV-1 and BVDV-2, have been identified with each genotype being either a CP or NCP biotype. BVDV-2 is generally more pathogenic and capable of causing severe haemorrhagic diarrhoea with high mortality rates. Within BVDV-1, subtypes have been identified (Luzzago et al. 2014). A third putative genotype, BVDV-3, an 'atypical' or 'HoBi-like' pestivirus, has recently been proposed (Bauermann, Flores & Ridpath 2012). To date, infection due to atypical BVDV has been reported in Asia, Europe and South America (Bauermann et al. 2013; Weber et al. 2014).

Pestiviruses infect a wide range of domestic animals and free-living ruminants. While cattle is the main host for BVDV, and the only bovid known to develop MD, BVDV has been reported in various domestic and free-ranging artiodactyls, including sheep and goats. Most cases of MD occur in cattle aged between six and 24 months (Radostits et al. 2007).

Infection due to bovine pestivirus is worldwide in distribution except in some European countries where it has been eradicated. In Australia, BVDV-1 is widespread while BVDV-2 has not been reported. In North America, both BVDV-1 and BVDV-2 are present. The distribution of the 'atypical' pestivirus is unclear but is known to be present in South America, Southeast Asia and Italy (Cortez et al. 2006; Decaro et al. 2011; Decaro et al. 2013; Ståhl et al. 2007; Weber et al. 2014).

BVD is an OIE-listed disease (OIE 2016d) but only BVD-2 is nationally notifiable in Australia (Department of Agriculture and Water Resources 2016). It is not categorised in the Emergency Animal Disease Response Agreement (EADRA) (AHA 2010).

4.4.2 Technical information

Pathogenesis

Pathogenesis depends on host factors as well as specific properties of the infecting BVDV-isolate (Radostits et al. 2007).

In naïve post-natal infections, transmission is more likely to be via the respiratory than the gastrointestinal tract (Ohmann 1983). Viraemia occurs 2–4 days after exposure and, in acute infections, the virus can be isolated from serum or leucocytes for 3–10 days post infection. The virus might initially replicate in the nasal mucosa and tonsils, before being transported to regional lymph nodes and later being disseminated throughout the body via leucocytes

(Potgieter 2004). Virus replication occurs in leukocytes of peripheral blood, fixed lymphoid tissues and bone marrow. The significance of other sites of replication is uncertain and although tissue tropism of strains might vary (Marshall, Moxley & Kelling 1996), the virus has been shown to cause disease by damaging epithelial tissues of the gastrointestinal, integumentary and respiratory systems (Ames 1986). BVDV is lymphotropic, leading to immunosuppression and increased susceptibility to concurrent disease (Walz et al. 2010).

Virulent strains of BVDV-2 have provoked a marked thrombocytopaenia, causing extensive haemorrhage. Leukopaenia was observed (Potgieter 2004).

In utero infections are most commonly caused by the NCP biotypes of BVDV (Potgieter 2004). Early reproductive losses occur, being a consequence of ovarian dysfunction, uterine inflammation or damage to the embryo (Grooms 2006). Infection at less than 100 days gestation may result in foetal death and resorption or later expulsion. From days 100 to 150, teratogenesis or ill thrift may result (Potgieter 2004). Congenital defects are thought to occur as a result of infection during organogenesis (Grooms 2006). Infection with NCP isolates before the development of immunocompetence at days 100–125 may result in immunotolerance to the specific infecting isolate and the development of PI animals. PI animals typically demonstrate reduced growth rates and increased susceptibility to other diseases (Potgieter 2004).

MD is associated with severe pathological lesions, and is a consequence of PI. There is a high mortality rate amongst PI calves during their first year of life. MD is believed to be the result of super infection of a PI animal with a CP isolate that is homologous to the initial persistent NCP isolate (Potgieter 2004), or alternatively through mutation of the persistent BVDV biotype, or recombination between NCP biotypes (OIE 2016c). The specific pathogenic mechanism involved might contribute to the disease being either early onset or late onset (Fritzemeier et al. 1997).

Clinical Signs

BVDV causes a wide variety of clinical signs. In seronegative immunocompetent cattle, it is characterised by inappetance, depression, fever and sometimes mild diarrhoea. Viraemia is transient and cases usually recover rapidly a few days later. Infection with BVDV-2 can result in fever, severe and sometimes haemorrhagic diarrhoea, severe lymphopenia, severe alimentary epithelial necrosis and lymphoid depletion (Kelling et al. 2002).

MD occurs in immunotolerant and PI cattle. After superinfection with a homologous CP biotype, clinical signs develop in two to three weeks. Small vesicle-ulcers develop in epithelial cells, resulting in erosions throughout the oral cavity, oesophagus, forestomach, abomasum, small intestines, caecum and colon. Ill thrift is common and death often follows, usually within two weeks of onset of clinical signs (Potgieter 2004).

Epidemiology

BVDV can be transmitted directly between animals, via the placenta to the foetus, mechanically by haematophagous flies, or venereally to cows. The virus can be excreted in nasal discharge, saliva, semen, faeces, urine, tears and milk during viraemia (Radostits et al. 2007). Transmission rates appear to be relatively low in acute post-natal infection and spread is often slow in yarded or housed cattle (Potgieter 2004). PI cattle are usually the main source of infection in a herd.

Live vaccines may damage the health of donors through immunosuppression and cause potentiation of other infections. Killed vaccines appear to be safe but sometimes do not provide

sufficient immunity to counter the viral challenge from PI animals. Modified live virus (MLV) vaccines are widely used in Canada and the United States, MLV vaccines used in North America are available as combination vaccines containing multiple attenuated pathogens including bovine herpesvirus-1 and BVDV-2. However, vaccination is not always fully effective in preventing viraemias and birth of PI calves (Rodning et al. 2010).

Diagnosis

The diagnosis of BVDV infection is complex because it depends on the purpose of the tests. Detection of BVDV in reproductive materials is difficult and requires greater care.

PI cattle are identified by isolating NCP virus in cell cultures from blood or serum. An immune-labelling method is used to detect virus growth in cultures. Tests for direct detection of viral antigen, using a capture ELISA or viral RNA in leukocytes are available. PI animals, which usually have no antibodies to BVDV, are confirmed by retesting at least three weeks later. The probe-based real-time or quantitative RT-PCR methods have high sensitivity, provided quality assurance protocols are closely followed. Semen can be tested for virus as well, either by virus isolation or by RT-PCR but requires care in preparation for shipping to laboratories for diagnosis.

However, bulls neither persistently infected nor viraemic may have persistently infected testicular tissues, e.g. testes and seminiferous tubules (Givens et al. 2009; Voges et al. 1998). Similarly, post-viraemic cows may continue to excrete BVDV from within avascular portions of ovarian follicle, granulosa cells and oocytes which cannot be attacked by cell-mediated immunity (Givens & Marley 2013).

Transient viraemia in cattle with BVD is difficult to detect due to the short viraemia period. MD is confirmed by isolating the CP biotype of BVDV from intestinal tissues, and by detecting NCP virus in blood.

The ELISA and the virus neutralisation test are the most widely used tests for antibody. Acute infection is best confirmed by demonstrating seroconversion using sequential paired samples from several animals in the group. The testing of paired (acute and convalescent) samples should be done a minimum of 14 days apart with samples being tested side by side (OIE 2016c).

Embryos and oocytes have been experimentally tested by PCR techniques but have not been validated for commercial use (Marley 2007; Marley et al. 2008).

4.4.3 Transmission in germplasm

Semen

Bulls excrete bovine pestivirus in semen during acute and transient infections and also when persistently infected. BVDV infection in adult bulls does not usually affect semen quality; however, semen with low sperm density and poor motility has been collected from healthy PI bulls (McGowan & Kirkland 1995). The virus can be isolated from whole semen, seminal plasma and washed cell fraction of fresh and frozen semen (Revell et al. 1988).

A rare event, possibly brought about by acute infection during pubescence, can result in persistent infection of the testes and thus strongly seropositive bulls that intermittently excrete virus in semen (Voges et al. 1998). This phenomenon has also been observed following vaccination with an attenuated virus (Givens et al. 2007).

Embryo

Embryo donor cows that are PI with BVDV also represent a potential source of infection, particularly as there are extremely high concentrations of BVDV in uterine and vaginal fluids. While oocytes without an intact zona pellucida have been shown to be susceptible to infection in vitro, the majority of oocytes remain uninfected with BVDV. Normal uninfected progeny has also been 'rescued' from PI animals by the use of extensive washing of embryos and in vitro fertilisation. Female cattle used as embryo recipients should always be screened to confirm that they are not PI (Givens & Waldrop 2004).

Consequently, the OIE Code Article 4.7.14 ranks BVDV in cattle as an IETS Category 3 disease, that is, a disease for which preliminary evidence indicates that the risk of transmission is negligible provided that the in vivo derived 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 (OIE 2016f).

With regards to in vitro produced embryos, the oocytes, follicular fluids and granulosa cells all represent sources of infection and in vitro culture represents a source of viral replication (Fray et al. 2000). IETS washing and trypsin treatment do not satisfactorily disinfect these embryos (Gard 2014; Gard, Givens & Stringfellow 2007, LaLonde and Bielanski, 2011) and in one study, transferring such embryos did result in transient infection of the recipient cows and some abortions but infection was not detected in the calves born (Bielanski et al. 2009).

Biological materials

Biological materials used for in vitro fertilisation techniques (bovine serum, bovine cell cultures) have a high risk of contamination with BVDV and should be screened (Gard 2014). Incidents of apparent introduction of the virus via such techniques have highlighted this risk. It is considered essential that serum supplements used in media be free of contaminants.

4.4.4 Current biosecurity measures

Australia has animal biosecurity measures for BVDV for bovine semen and in vivo derived embryos from Canada and the United States.

For bovine semen, the animal biosecurity measures require certification of donors tested according to either the CFIA export standards in Canada or the Certified Semen Services standards in the United States.

For in vivo derived embryos, the animal biosecurity measures require certification that the donors tested negative to the virus either by virus isolation or to an antigen capture ELISA.

4.4.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by BVDV:

- BVD is an OIE-listed disease but only BVD-2 is nationally notifiable in Australia.
- Each genotype is either a CP or NCP biotype. Of BVDV-1 and BVDV-2, BVDV-2 is generally more pathogenic and capable of causing severe haemorrhagic diarrhoea with high mortality rates.

- BVDV-1 is widespread globally and BVDV-2 has not been reported in Australia. In North America, both BVDV-1 and BVDV-2 are present. The atypical BVDV has been reported in Asia, Europe and South America.
- BVDV causes a wide variety of clinical signs, including inappetance, depression, fever and sometimes mild diarrhoea. BVDV also causes mucosal disease, where small vesicle-ulcers develop in epithelial cells, resulting in erosions throughout the gastro-intestinal tract, ill thrift and often death. Infection due to BVDV-2 is usually more severe than BVDV-1.
- The virus can cause persistent infection in cattle.
- Vaccination is not always effective in preventing viraemia in donors.
- BVDV can be transmitted via bovine semen, in vivo derived embryos and in vitro produced embryos.
- Although the diagnosis of BVDV infection is complex, there are a range of diagnostic tests and procedures available to adequately manage the risk of entry, establishment and spread of exotic BVDV via germplasm in Australia.

Conclusion

Based on the preceding factors, it was concluded that the overall risk of BVDV, particularly BVDV-2, associated with importing bovine germplasm has not changed, and animal biosecurity measures are required for bovine in vitro produced embryos.

The following biosecurity measures provide appropriate risk management.

For bovine in vitro produced embryos from Canada and the United States:

- At the time of each collection of oocytes, each female donor gave a negative result to one of the following tests for BVDV:
 - an antigen-capture ELISA on peripheral blood leucocytes
 - or
 - a monoclonal immunoperoxidase or other virus isolation test on blood or serum.
- AND
- If vaccinated, the donors were kept in a herd where all eligible animals including the donors were vaccinated against both BVDV-1 and BVDV-2 with a vaccine approved by the competent authority at least 30 days prior to collection of oocytes. The vaccine was administered as per manufacturer's instructions for vaccination and revaccination.
- AND
- The embryos were handled and treated in accordance with the IETS Manual, that is,
 - The embryos were washed at least ten times with at least 100-fold dilutions between each wash, and a fresh pipette was used for transferring the embryos through each wash.
 - and
 - Only embryos from the same donor were washed together, and no more than ten embryos were washed at any one time.

4.5 Enzootic bovine leucosis

4.5.1 Background

Enzootic bovine leucosis (EBL), caused by bovine leukaemia virus (BLV), is an infection of cattle characterised by a long incubation period and persistent lymphocytosis or lymphosarcoma. BLV can cause immune suppression in affected animals through multiple mechanisms and evidence suggests this impacts on cattle health and well-being, especially in regards to vaccine protection and susceptibility to other infectious diseases (Frie & Coussens 2015).

EBL occurs worldwide, particularly in dairy cattle, except in countries that have eradicated the disease. Prevalence of BLV infection in Canada and the United States is high and disease is widespread in dairy herds (Nekouei et al. 2015; USDA 2008) and less so in beef herds (Dargatz et al. 1998; Olaloku 2010).

Recent studies identified several different genotypes of BLV circulating (Moratorio et al. 2010).

Natural infections occur in cattle, water buffalo and occasionally in sheep kept in close contact with infected cattle (Green, Herbst & Schneider 1988). Cattle may be infected at any age, including the embryonic stage. Sheep are highly susceptible to experimental infection and goats less so.

In Australia, EBL has been eradicated from nearly all dairy herds, with provisional freedom being achieved in 2010 (Derrick 2010). EBL does occur in some beef herds in Australia and there is no national program for the eradication of disease from beef cattle. A survey of beef cattle aged between 18 and 36 months detected a seroprevalence of 0.22% in Brahman and Brahman-cross cattle and a herd prevalence of 6.8% (Ward 1995).

EBL is an OIE-listed disease (OIE 2016d) and is nationally notifiable in Australia (Department of Agriculture and Water Resources 2016).

4.5.2 Technical information

Pathogenesis

Infection does not cause detectable chronic viraemia but BLV does cause lifetime infection. The virus targets B-lymphocytes and sometimes infects T-cells, causing a chronic B-cell proliferative disease (Aida et al. 2013). Tumour transformation may occur after a variable incubation period. Infected animals develop a strong and persistent humoral response to the virus with most becoming latent carriers. Approximately 30% develop persistent lymphocytosis and others (0.1–10%) develop lymphosarcoma (OIE 2016c). Lymphocytosis is slow developing with clinical signs and death normally occurring in older cattle. Cattle with lymphosarcoma generally die within several months of developing clinical signs (EFSA Panel on Animal Health and Welfare 2015).

Clinical signs

The incubation period ranges from three months to several years. Most infected cattle remain clinically healthy throughout their lives with some developing benign persistent lymphocytosis. Others develop enlarged, firm superficial lymph nodes just under the skin at the shoulder or above the udder, rapid emaciation and death. Fewer than 5% of affected cattle develop lymphoma of the lymph nodes and internal organs. Clinical signs depend on the site of the tumours and are most frequently seen in cattle between three and eight years of age. Chronic

bloating, lameness and paralysis can occur if the tumours place pressure on the oesophagus or nerves (EFSA Panel on Animal Health and Welfare 2015).

Epidemiology

Transmission occurs when there is a transfer of blood lymphocytes containing the BLV provirus in their genome. Once introduced into the tissues of the recipient animal, the infected lymphocytes release the virus which then contaminates other lymphocytes of the new host (EFSA Panel on Animal Health and Welfare 2015).

Because there is little free virus in infected animals, transmission via urine, mucus or saliva is not possible unless contaminated with infected lymphocytes. Management procedures contributing to the exchange of blood between animals, such as multiple use of needles, contaminated surgical equipment, tattooing, ear tagging, dehorning, or common rectal palpation sleeves, can also transmit the virus. As little as 0.001 mL blood can contain enough infected lymphocytes to transmit the virus. Mechanical transmission by biting insects but not by ticks can occur (Morris et al. 1996; Radostits et al. 2007). While the virus can be shed in milk, there is inconclusive evidence that it can infect calves. Early work (Miller & van der Maaten 1979) suggests it can, but more recent work supports the hypothesis that colostrum contains protective antibodies, preventing infection in calves (Nagy, Tyler & Kleiboeker 2007). Congenital infection through the placenta has been reported and intra-uterine inoculation of infected leucocytes can cause infection in cows (Roberts et al. 1982).

Diagnosis

EBL is confirmed using tests on blood or milk. The serological tests are very accurate and capable of detecting very small quantities of EBL antibody. Tests can be performed on individual animals, on blood or milk samples (from the vat), or from a group of animals for export testing or other certification purposes (OIE 2016c).

Virus can be isolated by in vitro culture of peripheral blood lymphocytes from infected animals and identified using electron microscopy or by BLV antigen detection (OIE 2016c).

4.5.3 Transmission in germplasm

Semen

BLV virus has been demonstrated in bovine semen collected by massaging the pelvic genitalia of an old seropositive bull (Lucas et al. 1980) and proviral nucleic material demonstrated in nine of 173 semen samples collected from seropositive bulls at an artificial insemination Centre in Argentina (Dus Santos et al. 2007). It was suggested that the massage technique probably traumatised the genital tract, resulting in leucocyte contamination of the semen (Kaja & Olson 1982; Schultz et al. 1982).

Several authors report that sexual transmission during artificial insemination is unlikely (Belev et al. 1986; Miller & van der Maaten 1979; Roberts et al. 1982; Schultz et al. 1982; Tsutsumanski & Genov 1984). However, seroconversion occurred in sheep inoculated with semen spiked with 10^4 fetal lamb kidney cells persistently infected with the virus (Dus Santos et al. 2007). Semen appears to contain non-specific inhibitors of EBL virus, as incubating a mixture of infected lymphocytes with semen resulted in inactivation of the virus (Moskalik 1990; Roberts et al. 1982). Inseminating cows in a closed dairy herd free from EBL with over 1,000 semen units collected from seropositive bulls for over five years did not result in transmission (Monke 1986). Semen spiked with infected leucocytes can infect cows inseminated during the luteal phase of

the oestrus cycle, but not during the oestrus phase (Schultz, Buxton & Panangala 1982). It appears oestral mucus contains non-specific inhibitors of EBL virus as the virus was inactivated when incubating infected lymphocytes with oestral mucus (Moskalik 1990).

Embryos

Several studies have examined transfers of embryos from infected donors, some of which were inseminated with semen from infected bulls, into uninfected recipients. None of the recipients and none of the calves developed antibodies to BLV (Hare 1985; Thibier & Nibart 1987; Wrathall, Simmons & van Soom 2006). Washing of embryos is essential to remove possible infected lymphocytes in uterine flush fluids.

Consequently, the OIE Code Article 4.7.14 ranks EBL as an IETS Category 1 disease, that is, a disease for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the in vivo derived bovine embryos are properly handled and washed between collection and transfer (OIE 2016f).

There is a risk of infected blood contaminating oocytes collected by the OPU method. Bielanski, Maxwell and Simard (2000) reported that none of the in vitro produced embryos, washed according to IETS Manual, tested positive to proviral DNA, after contaminating the maturation medium, the semen used for fertilising the oocytes, and the embryo culture medium with BLV. Because little is known about the association of BLV and other retroviruses with sperm cells and oocytes, the authors advised using semen from bulls free from BLV.

4.5.4 Current biosecurity measures

Australia has animal biosecurity import measures for EBL for bovine semen from Canada and the United States. These measures are as follows:

Canada

- Certification of donors tested according to Canadian export standards.

United States

- USDA:APHIS certification that donors originate from herds free from EBL and are tested free of EBL antibodies
- OR
- semen is tested free of BLV antigen.

4.5.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by BLV:

- EBL is an OIE-listed disease and is nationally notifiable.
- Australian dairy herd achieved freedom on 31 December 2016 but is present in some beef herds. EBL is widespread in Canada and the United States with high prevalence of BLV infection in dairy herds and less so in beef herds.
- Most infected cattle remain clinically healthy throughout their lives with some developing benign persistent lymphocytosis. Others develop enlarged, firm superficial lymph nodes just under the skin at the shoulder or above the udder, rapid emaciation and death.

- Transmission and spread is via infected lymphocytes.
- There is evidence that BLV can be transmitted via bovine semen containing infected lymphocytes.
- There are a range of diagnostic tests and procedures available to adequately manage the risk of entry, establishment and spread of EBL via germplasm in Australia.
- Where the in vivo derived bovine embryos are properly handled and washed to remove infected lymphocytes between collection and transfer, the risk of transmission is negligible. Provided semen from bulls free from BLV is used, the risk of transmission via in vitro produced embryos is negligible.

4.5.6 Conclusion

Based on the preceding factors, it was concluded that the overall risk of BLV associated with importing bovine germplasm has not changed, and animal biosecurity measures are required for bovine in vitro produced embryos.

The following biosecurity measures provide appropriate risk management.

For bovine in vitro produced embryos from Canada and the United States:

- Only semen certified or eligible for export to Australia was used to fertilise the oocytes. Evidence was provided by the Team Veterinarian to the Veterinary Authority for endorsement.

AND

- The embryos were handled and treated in accordance with the IETS Manual, that is,
 - The embryos were washed at least ten times with at least 100-fold dilutions between each wash, and a fresh pipette was used for transferring the embryos through each wash.
 - and
 - Only embryos from the same female donor were washed together, and no more than ten embryos were washed at any one time.

4.6 Epizootic haemorrhagic disease

4.6.1 Background

Epizootic haemorrhagic disease (EHD) is an insect-borne viral disease of ruminants, characterised by haemorrhagic fever in deer and rarely bluetongue-like illness in cattle.

There are seven serotypes of EHD virus (EHDV) distributed worldwide that may be differentiated on the basis of topotype or nucleotype but there is not yet a widely accepted consensus on the exact number of serotypes (Anthony et al. 2009).

EHDV is capable of infecting wild and domestic ruminants but historically clinical disease was seen mostly in wild cervids, particularly white-tailed deer (*Odocoileus virginianus*) of North America. Severe clinical signs have been observed in cattle infected by the Ibaraki strain (EHDV-2) (Omori et al. 1969) and the serotypes 6 and 7 (Temizel et al. 2009; Yadin et al. 2008).

The distribution of EHD is limited to the distribution of competent *Culicoides* vectors. According to the reported cases, EHDV lies approximately between latitudes 49° North and 35° South. Consequently EHDV has been reported in North and South America, Australia, Asia and Africa, and more recently in countries surrounding the Mediterranean Basin including Morocco, Algeria, Tunisia, Israel, Jordan and Turkey; but it has not been reported in Europe.

Six non-pathogenic serotypes (1, 2, 5–8) of EHDV have been identified in Australia from insects or sentinel cattle but clinical disease has not been reported in Australia (Weir et al. 1997).

In North America the distribution of EHDV (serotypes 1, 2 and 6) tend to reflect the distribution and activity of *Culicoides* sonorensis with most cases of EHD occurring in the late summer and autumn (Stallknecht & Howerth 2004). However, there are a few reports of EHD as far north as New Jersey in the United States and western and southern Canada that are out of the normal range for this vector species, suggesting different *Culicoides* vectors are also involved (Shapiro et al. 1991; Stallknecht & Howerth 2004).

EHD is an OIE-listed disease (OIE 2016d). In Australia, clinical EHD is nationally notifiable but is not categorised in the EADRA (AHA 2010; Department of Agriculture and Water Resources 2016).

4.6.2 Technical information

Pathogenesis

Viraemia is detectable two to three days post-infection and usually lasts less than three weeks but may persist for eight weeks (EFSA 2009). Viraemia from EHDV lasting 20 days in cattle and 11 days in experimentally infected sheep has been reported (Uren 1986).

After infection of the skin through the bite of an infected vector, the virus replicates in a regional lymph node before further replication in secondary sites such as lungs and spleen. Dissemination to a variety of tissues, including the alimentary tract and muscles occurs by association of the virus with red blood cells and endothelial cells, even in the presence of high antibody titres. In animals that show clinical signs, EHDV causes widespread vasculitis, accompanied by oedema and haemorrhages in many tissues including the tongue, salivary glands, walls of the forestomachs, aorta and myocardium of the left ventricle (MacLachlan & Osburn 2004). In Ibaraki disease degeneration of the muscles of the oesophagus, larynx, pharynx

and tongue may occur but lesions are not seen in other striated muscles. Petechial haemorrhages may be present in epicardium, myocardium and endocardium, liver, spleen, bladder, uterus, intestines and lymph nodes. Focal interstitial nephritis may occur in kidneys (Inaba 1975; Kitano 2004).

Clinical signs

EHD causes an often fatal haemorrhagic disease in the North American white-tailed deer (*Odocoileus virginianus*).

The clinical signs of EHD in cattle are stomatitis and coronitis, similar to signs seen with bluetongue. A notable feature of EHD outbreaks in Republic of South Africa in 1995–97 was a 42% drop in milk production in affected dairy herds (Barnard, Gerdes & Meiswinkel 1998). EHDV is generally non-pathogenic in sheep and goats. Sheep do not respond serologically to infection by some EHDV serotypes (Gard, Melville & Shorthose 1989).

Epidemiology

EHD is non-contagious, being biologically transmitted by *Culicoides* species. Transmission requires a population of competent adult vectors, favourable climatic conditions for virus amplification in the vectors and sufficient viral load to initiate infection and amplification in cattle.

The distribution of EHD depends on the presence and density of animal reservoirs, amplifying hosts such as wild ruminants and cattle, and suitable *Culicoides* vectors in sufficient numbers.

In infected herds, most animals would have already been infected by at least one serotype and be immune to that serotype. However, they would still be susceptible to other serotypes. Susceptible animals are most likely to become infected during the late summer/early autumn period and remain infected for up to eight weeks.

No vaccine is available for EHDV.

Diagnosis

Accurate diagnosis relies on virus isolation and antibody detection. EHDV is isolated by cell culture. Serotypes of EHD are recognised and are differentiated by serum neutralisation tests, despite cross-reactions between some serotypes, and nucleic acid tests. All EHD serotypes share group antigens detectable by CFT and AGID test. Recently, ELISAs have been developed to detect serum antibody to EHDV (Mecham & Jochim 2000). The OIE Manual recommends the specific monoclonal antibody-based competitive ELISA (cELISA) for serology.

Assays for identification of EHDV in field samples include virus isolation in cell culture, EHD serogroup-specific reverse-transcription polymerase chain reaction (RT-PCR) tests, and competitive (antigen-capture) and sandwich enzyme-linked immunosorbent assays (ELISAs). Serotype-specific RT-PCR assays have been developed for serotype identification of cell culture isolates. Isolates may also be identified by high throughput sequencing or virus neutralisation tests (OIE 2016c).

4.6.3 Transmission in germplasm

Semen

There is no evidence of EHDV infecting semen of bulls two to four years old (Gard, Melville & Shorthose 1989). However, experimental and epidemiological studies demonstrate that virulent

strains of the closely related BTV can infect semen of mature bulls but not young bulls (Melville & Kirkland 1994). Acute fever in bulls affects semen quality and clinically affected bulls are likely to be removed and poor quality semen rejected. The possible presence of EHDV in semen is likely to correspond to its viraemic period, as for BTV.

Embryos

There is no information regarding infection of embryos with EHDV and according to the OIE Code Article 4.7.14, the IETS has not yet categorised EHD.

4.6.4 Current biosecurity measures

Australia has animal biosecurity measures for EHDV for bovine semen but not for bovine in vivo derived embryos from Canada and the United States. These measures were implemented when EHD was not an OIE-listed disease, that is, when it was determined to be a disease not of significance to international trade and also when there was regular BTV/EHD surveillance in North America.

The animal biosecurity measures require certification of semen donors in EHD/BTV seasonally free zones, negative antibody test or negative antigen test.

4.6.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by EHDV:

- EHD is now an OIE-listed disease and clinical EHD is nationally notifiable in Australia.
- EHD is regularly reported in the United States and occasionally in Canada. There is now no surveillance for EHDV to enable the USDA: APHIS to classify the EHDV/BTV low incidence States and no semen collection centres in the Okanagan Valley in British Columbia, Canada.
- An insect-borne viral disease, EHDV activity increases when vector activity peaks during late summer and autumn.
- Clinically, EHD is characterised by cyanosis of mucous membranes with widespread haemorrhages and oedema in deer, especially white-tailed deer and, on rare occasions, cattle. It can cause mortalities in white-tailed deer.
- All ruminant species are susceptible to infection and most species are generally subclinically infected.
- There is insufficient information to assess the risk of infection with EHDV of, and hence the transmission via, bovine semen and in vivo derived and in vitro produced embryos.
- Diagnostic testing of donors using properly validated tests provides adequate risk management against risk of entry, establishment and spread of EHDV via germplasm in Australia.

4.6.6 Conclusion

Based on the preceding factors, it was concluded that biosecurity measures are required for bovine in vitro produced embryos. The following biosecurity measures provide appropriate risk management.

For bovine in vitro produced embryos from Canada and the United States:

- Blood samples drawn from each donor
 - were subjected to the cELISA test to detect antibodies to the EHDV group between 28 and 60 days after each collection for this consignment with negative results
 - or
 - were subjected to an agent identification test on a blood sample taken on the day of each collection with negative results.
- OR
- All oocytes donors were located in a country free or seasonally free from EHDV, as recognised by Australia* at least 60 days prior to, and at the time of, collection of oocytes.

[Australia recognises Canada as a country seasonally free from EHDV without testing between 1 January and 15 May, except Okanagan Valley of British Columbia]

4.7 Foot and mouth disease

4.7.1 Background

Foot and mouth disease (FMD) is a highly contagious, but rarely lethal, vesicular disease affecting mainly Artiodactylae. Of the domestic livestock species, FMD virus infects cattle, buffalo, pigs, sheep, goats and deer but is generally most severe in cattle and pigs. Several wild cloven-hoofed species are also susceptible, as are Asian elephants, hedgehogs and some rodents. Seven serotypes of FMD virus are recognised—A, O, C, SAT1, SAT2, SAT3 and Asia 1.

This disease is enzootic in parts of Asia, Africa, the Middle East and South America. North America, Australia and New Zealand are free from FMD. Some European Union (EU) Member States have in recent years reported outbreaks of FMD, in particular, the United Kingdom in 2007 and Bulgaria in 2011.

FMD is an OIE-listed disease for which the OIE established official recognition of the sanitary status countries and zones (OIE 2016d). In Australia, it is nationally notifiable and is classed as an EADRA Category 2 disease, that is, a disease that has the potential to cause major national socio-economic consequences through significant international and domestic market disruptions and very severe production losses in the affected livestock industries (AHA 2010; Department of Agriculture and Water Resources 2016).

4.7.2 Technical information

Pathogenesis

The primary site of infection and initial viral replication is in the epithelium of the pharynx and dorsal soft palate. No lesions are produced at this stage and the virus can persist in the pharynx for more than 28 days. The virus then enters the bloodstream via regional lymph nodes and spreads to secondary sites. In particular, the keratinised stratified squamous epithelial cells in tissues are major sites for FMD virus replication and formation of vesicular lesions. These cells are found in the tongue, teat, coronary band, heel bulb and the interdigital cleft of cloven-hoofed animals. Other tissues, particularly the myocardium, might harbour the virus without showing lesions though virulent infections have resulted in fatal myocarditis in young animals (Alexandersen et al. 2003).

Clinical signs

FMD virus typically enters and infects animals by ingestion, inhalation, through a skin break or artificial insemination (Callis 1996). Once in the bloodstream, the virus is widely distributed, probably in mononuclear cells, irrespective of the portal of entry (Yilma 1980). After an incubation period ranging from 1–21 days, but averaging 3–8 days, vesicles develop in the epithelium of the mouth and feet and, to a lesser extent, the teats. Excretion of FMD virus occurs one to four days before onset of clinical signs with high levels of virus in secretions and respiratory aerosols (Thomson & Bastos 2004).

Lesions indicative of ruptured vesicles undergoing healing and chronic lameness are clinical signs typically seen in cattle recovering from FMD.

Epidemiology

FMD virus can be transmitted between herds or flocks, countries and continents in several ways, with direct contact between infected and susceptible animals being the most important. Widespread transmission has resulted from the exhalation of infective aerosols from pigs.

Indirect transmission is usually via fomites. FMD virus has entered countries in infected meat scraps, and hay. The virus retains infectivity in the environment for several weeks, longer in the presence of organic matter (AHA 2002). Humans can transfer infection through contaminated clothing, via virus on their hands or in their nostrils.

Up to 80% of cattle and 40% of sheep may become carriers after clinical recovery from FMD (Moonen et al. 2004; Orsel et al. 2007; Parida et al. 2008). The pharyngeal and upper oesophageal regions are sites for persistent infections which generally last four to five months but have lasted as long as 42 months (AHA 2002). The virus does not appear to pass into the salivary secretions of carriers (Graves et al. 1971). The role of carriers in the transmission of FMD remains uncertain since transmission from carriers to susceptible animals was not conclusively demonstrated under experimental conditions (Samara & Pinto 1983). However, epidemiological evidence suggests that transmission between asymptomatic carrier buffaloes and domestic cattle have occurred under field conditions with clinical disease occurring several months after the removal of buffaloes (Bastos et al. 1999).

Vaccinated animals may be infected despite protection against disease and may even become carriers (Kitching 2002). However, where large numbers of cattle were systematically vaccinated with good quality vaccines, FMD disappeared despite large sentinel populations of calves and unvaccinated sheep and pigs. While a low number of carriers most likely persisted, they did not hamper the eradication of the disease (Sutmoller & Casas 2002).

Strain specific antibodies appear in the serum seven to ten days post infection. Antibodies have little effect on the carrier status of the animal as the virus persists in the pharynx despite circulating antibodies (Alexandersen et al. 2003).

Specimens suitable for rapid laboratory confirmation of FMD include samples of affected tissues (unfixed) and fluids from vesicles for virus isolation and serum for serology. A number of diagnostic tests are available for detecting and identifying whole virus, virus antigen and viral antibodies (OIE 2016c).

Diagnosis

Diagnosis of FMD is by virus isolation or by the demonstration of FMD viral antigen or nucleic acid in samples of tissue or fluid. Detection of virus-specific antibody can also be used for diagnosis, and antibodies to viral non-structural proteins can be used as indicators of infection, irrespective of vaccination status (OIE 2016c).

4.7.3 Transmission in germplasm**Semen**

Semen from FMD infected bulls can contain virus. The virus was detected in the seminal fluid of infected cattle and boars (McVicar et al. 1977). It was also isolated from the semen of 12 of 16 experimentally infected bulls for up to ten days post-infection. The infection resulted in poor quality semen in four bulls (Cottral, Gailunas & Cox 1968). In another study, viral antigen was detected in bovine semen for up to 60 days post infection (Gajendragad et al. 2000). Infective

virus was also isolated from the semen and sheath wash from a wild seropositive buffalo showing no clinical signs of FMD (Bastos et al. 1999). Virus present in the preputial orifice may contaminate semen.

FMD virus can be present on the prepuce and coat of vaccinated bulls (Sellers et al. 1969; Sellers, Herniman & Gumm 1977) and may contaminate semen during ejaculation.

Embryos

FMD virus can contaminate embryos of infected donor cows. Low levels of virus were isolated from vaginal swabs, uterine flush fluids and uterine sediments of cows showing acute signs of FMD, but high levels of virus were detected in some ovarian tissues and follicular fluids of these cows. However, FMD virus could not be isolated from the 169 in vivo derived embryos with intact zona pellucida collected from these cows after being washed 10 times without trypsin (McVicar et al. 1986). Washing porcine embryos infected in vitro was not completely effective nor was washing hatched bovine in vivo derived embryos (Singh et al. 1986).

Consequently the OIE Code Article 4.7.14 ranks FMD in cattle as an IETS Category 1 disease, that is, a disease for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the in vivo derived bovine embryos are properly handled and washed between collection and transfer (OIE 2016f).

Washing bovine in vitro produced embryos according to the IETS Manual recommendations is not effective in removing FMD virus (Marquant-Le Guienne et al. 1998).

4.7.4 Current biosecurity measures

Australia regularly reviews its FMD policy given the advances in the control and eradication of the disease, including vaccination. Australia's animal biosecurity measures for semen and embryos require certification of OIE recognition of country freedom of FMD without vaccination.

4.7.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by the FMD virus:

- FMD is an OIE-listed disease and is nationally notifiable in Australia.
- FMD virus is enzootic in parts of Asia, Africa, the Middle East and South America. North America, Australia and New Zealand and most of Europe are free from FMD.
- It is highly infectious in domestic livestock, especially cattle, buffalo, pigs, sheep, goats and deer.
- Vaccination and serological testing of donors or donor herds do not provide adequate risk management against risk of entry, establishment and spread of FMD in Australia.
- FMD has the potential to cause major national socio-economic consequences through very serious international trade losses, national market disruptions and very severe production losses in the livestock industries that are involved.
- There is evidence that FMD virus can be transmitted via semen, in vitro produced embryos and those washed in vivo derived embryos with damaged zona pellucida or that have hatched.

4.7.6 Conclusion

Based on the preceding factors, it was concluded that the overall risk of FMD virus infection associated with importing bovine germplasm has not changed and is similar for bovine in vitro produced embryos. Hence animal biosecurity measures that applied to bovine semen and bovine in vivo derived embryos will also apply for bovine in vitro produced embryos from Canada and the United States.

The following biosecurity measures provide appropriate risk management.

For bovine in vitro produced embryos from Canada and the United States:

- Donors showed no clinical signs of disease on the day(s) of oocyte collection and for 30 days after.
- Donors resided in Canada/the United States for at least 90 days prior to oocyte collection for this consignment.
- At the time of, and for 30 days after, each oocyte collection for this consignment, Canada/the United States was officially recognised by the OIE and Australia as an FMD free country where vaccination is not practised.

4.8 Infectious bovine rhinotracheitis and infectious pustular vulvovaginitis

4.8.1 Background

Bovine herpesvirus (BoHV-1) causes a complex of disease syndromes including infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV) and infectious pustular balanoposthitis. Other syndromes depend on the tissues infected and subtype of BoHV-1 but include: respiratory tract infections, eye infections, abortions, genital infections, and a generalised infection of newborn calves.

There are three subtypes, BoHV-1.1, BoHV-1.2a and BoHV-1.2b. BoHV-1.1 is commonly associated with respiratory disease, and BoHV-1.2a and BoHV-1.2b with genital disease (Muylkens et al. 2007). Abortions have occurred with BoHV-1.1 and 1.2a (Miller et al. 1991), and respiratory disease with BoHV-1.2a and BoHV-1.2b. BoHV-1.2b is generally less virulent than BoHV-1.1, typically causes only subclinical infection or very mild clinical signs, and has not been associated with abortions (Muylkens et al. 2007). The virus is enveloped and is therefore sensitive to many disinfectants, especially solvents (Straub 1990).

BoHV-1 infects domestic and wild cattle of all ages and has been isolated from water buffalo with both respiratory and genital disease (Brake & Studdert 1985; Nandi et al. 2009). Other ruminants may be infected with BoHV-1 but are not significant in the transmission of virus.

BoHV-1 occurs in nearly every cattle-raising country. All subtypes are present in Europe (except in countries/zones that have eradicated BoHV-1, e.g., Austria, Denmark, Finland, Sweden, Italy (Province of Bolzano), Switzerland, Norway and parts of Germany (the 'Oberfranken' and 'Oberpfalz' districts of Bavaria) and North America.

Only subtype 1.2b has been isolated in Australia (Gu & Kirkland 2003). IBR was a notifiable disease in parts of Australia for many years but is not on the current (2014) nationally notifiable disease list. However, it is notifiable in Victoria. IBR infections in Australia are generally subclinical (Beveridge 1986), unlike subtypes 1.1 and 1.2a which can cause severe respiratory illness (Spilki et al. 2004, Kaashoek et al. 1996). Australian isolates has not been observed to cause abortion under natural or experimental conditions (Allan, Dennett & Johnson 1975; Young 1993; AHA 2016a). The lack of evidence of more virulent subtypes and a predominance of pasture-based grazing means that disease due to IBR is rare in Australia (AHA 2016a). Based on past history (that is, no abortions implicating BoHV-1), past surveys, passive surveillance for virulent or abortigenic pathogens of the respiratory and reproductive systems, diagnostic testing of live breeder cattle and bovine semen for export to certain countries, and the lack of vaccination for IBR/IPV in breeding dairy and beef herds, Australia is assessed to be a country free from BoHV-1.1 and BoHV-1.2a (AHA 2016a).

IBR/IPV is an OIE-listed disease (OIE 2016d). IBR/IPV, along with several other OIE listed diseases such as bovine viral diarrhoea (BVD), bovine genital campylobacteriosis and trichomonosis, is recognised in the OIE Code Chapter 4.6 as a significant reproductive disease of bovine germplasm for which risk management is recommended, irrespective of the disease status in the exporting and importing countries. This disease, along with BVD, is similarly recognised in the OIE Code Chapter 4.7: Collection and processing of in vivo derived embryos

from livestock and equids (trypsin treatment) and 4.8: Collection and processing of in vitro produced embryos/oocytes from livestock and horses (semen collected according to OIE Code).

4.8.2 Technical information

Pathogenesis

The natural portal of BoHV-1 entry is via the mucous membranes of either the upper respiratory tract or genitals. After an incubation period of 2-4 days, there is massive viral replication in the epithelial cells and BoHV-1 then spreads from cell to cell (Muylkens et al. 2007; Rebordosa et al. 1996). New progeny BoHV-1 are shed for about 5-10 days in nasal mucous at high excretion titres and rapidly disseminate infection within a cattle herd. Direct nose-to-nose contact is the common mode of transmission of BoHV-1 (Muylkens et al. 2007). Airborne transmission by aerosol was demonstrated experimentally over short distances (Mars et al. 2000).

Besides local dissemination of BoHV-1, there is occasionally viraemia with systemic spread to other organs (Wyler, Engels & Schwyzer 1989). BoHV-1 is thought to infect neurones via nerve endings in the mucosae and ascend towards the central nervous system (Engels & Ackermann 1996). The virus prefers the trigeminal nerve, localising in the trigeminal ganglion where a latent infection is established. Once infected, animals become lifelong carriers of the virus (Enquist et al. 1998). Reactivation from latency can occur in response to natural stimuli such as parturition or stress during transportation (Thiry et al. 1985; Thiry et al. 1987). It can also occur after corticosteroid treatment culminating in recurrent virus transmission to uninfected animals generally without clinical signs (Sheffy & Davies 1972).

Genital infection requires direct contact at mating or can occur via virus-contaminated semen (Kupferschmied et al. 1986).

Clinical signs

Associated with a wide variety of clinical syndromes, BoHV-1 usually infects either the genital tract, causing pustular vulvovaginitis in cows and clinical balanoposthitis in bulls, or the upper respiratory tract, causing purulent nasal discharge, conjunctivitis and sometimes coughing. Other clinical signs include fever, depression, inappetance, abortions, and reduced milk yield (Muylkens et al. 2007).

Stress, such as that caused by intercurrent disease, transportation, cold, overcrowding, vaccination or corticosteroid treatment, can reactivate latent infection and cause the virus to be shed intermittently into the environment (Winkler, Doster & Jones 2000).

While most infections are subclinical, the main biological effects of genital BoHV-1 is pustular vulvovaginitis, shortened oestrus cycle, temporary infertility, drop in milk yield in lactating cows, and conjunctivitis. Abortion is sometimes a common feature of BoHV-1.2a infection. BoHV-1.2b can cause IBR and IPV but it is relatively benign and is not foetopathic or abortigenic (van Oirschot 1995).

Epidemiology

In common with other herpesviruses, BoHV-1 establishes a lifelong latent infection primarily, but not exclusively, in nerve ganglia (van Engelenburg et al. 1995).

Respiratory infections are usually transmitted by aerosol whilst genital infections are transmitted venereally. BoHV-1 is regarded as a highly contagious virus and severe outbreaks

have been reported in cattle kept in close confinement. Most infections run a subclinical course. Incubation period is usually two to four days. Uncomplicated cases last five to ten days, sometimes up to 20 days. With the respiratory form, the virus enters via the nose and replicates in the mucous membranes of the upper respiratory tract, disseminates to the conjunctivae, then moves to, and becomes latent in, the trigeminal ganglion. With the genital form, the virus replicates in the mucous membranes of the vagina or prepuce and moves to, and becomes latent in, the sacral ganglia. BoHV-1 latency in cattle is lifelong (Muylkens et al. 2007).

BoHV-1 is excreted from vaginal and nasal secretions of genitally infected cattle. Infection due to venereal transmission seems to remain more locally restricted than with intranasal transmission, but this depends largely on the strain of BoHV-1 involved. Excretion is usually intermittent over the lifetime of an infected animal and generally depends on its stress status (Muylkens et al. 2007).

Up to 100% of animals in a herd can become infected with BoHV-1 (Hage et al. 1996). Surveys have shown up to 96% of bulls infected with BoHV-1 (Radostits et al. 2007).

Live attenuated and killed vaccines are available to provide protection against clinical disease and reduce virus shedding (Platt et al. 2006; Xue et al. 2010). Marker vaccines are available but apparently not used in Canada and the United States. Intensive vaccination programs can reduce disease prevalence, especially within-herd prevalence (OIE 2016c). Modified live vaccines containing BoHV-1 are widely used in breeder cattle in Canada and the United States. However, BoHV-1 strains, including vaccine strains, continue to be isolated from diseased animals or fetuses after vaccination, indicating latent infection (Fulton et al. 2015, Fulton et al. 2016).

Diagnosis

The virus isolation test can be used to isolate BoHV-1 from nasal swabs taken during the acute phase of the infection. The virus neutralisation test and various ELISA tests, commonly used for antibody detection, are OIE-prescribed tests for international trade.

Detection of latently infected animals relies on post-mortem detection of the virus in tissues such as the trigeminal ganglia as current serological methods do not always identify latently infected animals (Puentes et al. 2016)

Aliquots of semen can be tested for the virus by a virus isolation test with two passages in cell culture or PCR. Because semen of infected bulls are infected sporadically or have very low titres of BoHV, it is essential that at least three straws from each batch be transported frozen or chilled to the laboratory and tested. Detection of BoHV-1 in semen by PCR is generally more sensitive than virus isolation, even so, it recommended PCR amplification be duplicated for each DNA preparation. Because the seminal fluid contains enzymes and other factors that are toxic to the cells and inhibit viral replication for the virus isolation test, it is necessary to treat the semen to remove the toxic factors (OIE 2016c; van Oirschot 1995).

Research on in vitro produced embryos and ova has included testing ova and follicular fluids by PCR techniques (D'Angelo et al. 2009; Marley et al. 2008). As yet the OIE Manual has not provided guidelines for PCR on embryos/oocytes for international trade purposes. Vaccination generally induces strong humoral but weak cell-mediated immune responses (van Drunen Little-van den Hurk, S, 2006). However, research has shown a high negative correlation between

neutralising antibody titres and virus shedding, with serum neutralising titres as an indicator of protection from disease and virus infection (Van Donkersgoed et al. 1994).

The OIE Manual provide for identification of BoHV-1 virus from nasal, ocular or genital swabs.

4.8.3 Transmission in germplasm

Semen

BoHV-1 is recognised to be the most common viral pathogen found in semen (Abraham, Prudovsky & Ayalon 1975; Autrup & Bitsch 1978; Deas & Johnston 1973). BoHV-1 was a common cause of infections in donor bulls in European semen collection centres which was spread by artificial insemination to several herds before control measures were introduced (Gössler & Paulsen 1975).

BoHV-1 can replicate in the preputial and penile mucosae and be isolated from semen and preputial washings of bulls (Bitsch 1973). Not all bulls with respiratory BoHV-1 infections have infected semen. Some may only sporadically shed BoHV-1 in semen (de Gee, Wagter & Hage 1996). Stress can reactivate the virus, resulting in intermittent shedding of virus in the seminal plasma (van Oirschot 1995).

Seronegative bulls may also shed BoHV-1 in their semen (Hage et al. 1998). In addition, semen can be contaminated by a primary preputial infection before antibodies are produced. BoHV-1 has been detected in the semen of a bull six weeks before it seroconverted.

Embryo

It was observed that enveloped viruses such as BoHV-1 can adhere firmly to the zona pellicula so that thorough washing could not remove the virus. However, exposure to the enzyme trypsin has resulted in the removal of the enveloped viruses from bovine in vivo derived embryos (Singh et al. 1982b; Singh et al. 1983; Singh 1987; Stringfellow et al. 1990). Even so, field data on embryos exported in France over many years indicated that none of the recipients of embryos seroconverted even though some of the donors from which embryos had been collected were positive for antibodies (Thibier & Nibart 1987).

Consequently, the OIE Code Article 4.7.14 ranks IBR/IPV (trypsin treatment required) as an IETS Category 1 disease, that is, a disease for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the in vivo derived bovine embryos are properly handled and washed between collection and transfer (OIE 2016f).

Oocytes from donors infected with BoHV-1 yielded embryos and follicular fluids that tested positive for BoHV-1 (Bielanski & Dubuc 1994). IETS washing and trypsin treatment was not successful in removing, but was successful in reducing, BoHV-1 in all bovine in vitro produced embryos (Bielanski et al. 1997).

4.8.4 Current biosecurity measures

Australia has animal biosecurity measures for BoHV-1 for bovine semen and in vivo derived embryos from Canada and the United States.

For bovine semen, the animal biosecurity measures require certification of donors in BoHV-1 free herds or establishments, held in isolation and negative antibody tests or aliquots of semen with negative antigen tests as defined in the OIE Code.

For in vivo derived embryos, the animal biosecurity measures require certification that the embryos were treated with trypsin during the washing process as described in the latest edition of the IETS Manual.

4.8.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by BoHV-1:

- IBR/IPV is an OIE-listed disease .
- The OIE Code recommends risk management for bovine germplasm to avoid transmission to recipient animals and their progeny.
- BoHV-1 occurs in nearly every cattle-raising country. All subtypes are present in North America but evidence indicates only subtype 1.2b is present in Australia.
- The virus usually infects either the genital tract, causing pustular vulvovaginitis in cows and clinical balanoposthitis in bulls, or the upper respiratory tract, causing purulent nasal discharge, conjunctivitis and sometimes coughing. Other clinical signs include fever, depression, inappetence, abortions, and reduced milk yield.
- BoHV-1 can infect, and be transmitted via bovine semen, in vivo derived embryos not treated with trypsin, and in vitro produced embryos.
- Washing and trypsin treatment as recommended in the IETS Manual reduces BoHV-1 in bovine in vitro produced embryos.
- Most valuable breeding stock are vaccinated with modified live vaccines containing BoHV-1 in Canada and the United States.
- Diagnostic testing of semen donors, or semen using properly validated tests and procedures as recommended in the OIE Manual provide adequate risk management against risk of entry, establishment and spread of IBR/IPV via bovine semen in Australia. This includes the virus isolation test on aliquots of semen.
- The OIE Manual provide for identification of BoHV-1 virus from nasal, ocular or genital swabs.

4.8.6 Conclusion

Based on the preceding factors, it was concluded that the overall risk of BoHV-1 associated with importing bovine germplasm has not changed, and animal biosecurity measures are required for bovine in vitro produced embryos.

The following biosecurity measures provide appropriate risk management.

For bovine in vitro produced embryos from Canada and the United States:

- For all oocytes collected from donors:
EITHER
 - The donor came from an IBR/IPV free herd as defined in the current OIE Codeor

- The donor was subjected, with negative results, to a serological test for IBR/IPV on blood samples collected at least four weeks after each oocyte collection.

OR

- The donor was kept in a herd where all eligible animals including the donor were vaccinated against IBR/IPV with a vaccine approved by the competent authority at least 30 days prior to collection of oocytes. The vaccine was administered as per manufacturer's instructions for vaccination and revaccination and
- A vaginal mucus sample[#] was collected from the donors immediately prior to washing/disinfecting the vagina in preparation for oocyte collection and tested for bovine herpesvirus-1 by the qRT-PCR for bovine herpesvirus-1 with negative results.
[#]Double guarded culture swabs, appropriately prepared for testing samples, were used to hygienically collect samples of vaginal mucus from the donors.

AND

- The embryos were handled and treated in accordance with the current IETS Manual. That is:
 - the embryos were washed at least ten times with at least 100-fold dilutions between each wash, and a fresh pipette was used for transferring the embryos through each wash
 - the embryos were treated with trypsin and
 - only embryos from the same donor were washed together, and no more than ten embryos were washed at any one time.

4.9 Lumpy skin disease

4.9.1 Background

Lumpy skin disease (LSD) is an infectious viral disease of cattle that often occurs in epidemic form. The disease is characterised by the eruption of nodules in the skin which may cover the whole of the animal's body.

The LSD, sheep pox and goat pox viruses belong to the genus Capripoxvirus of the family Poxviridae (Buller et al. 2005). These viruses are morphologically indistinguishable from each other, but are adapted to different host species. The viruses are difficult to distinguish serologically, and cross protection does occur (Kitching 1983).

LSD mainly affects cattle, with occasional cases in Asian water buffalo (*Bubalis bubalis*). *Bos taurus* cattle are generally more susceptible than *Bos indicus* (zebu) cattle; Jersey, Guernsey, Friesian and Ayrshire breeds being particularly susceptible (Davies 1991). African buffalo have shown serological evidence of infection in endemic areas although clinical disease has not been observed (Davies 1982). They also appear to be relatively resistant to experimental infection (Young, Basson & Weiss 1970).

LSD is endemic in many African and Asian countries, and it is rapidly spreading throughout the Middle East, and parts of Europe and Russia (OIE 2016b).

LSD is an OIE-listed disease (OIE 2016d).

LSD has not occurred in Australia (AHA 2009). In Australia, LSD is nationally notifiable and is classed as an EADRA Category 3 disease. An EADRA category 3 disease is, a disease that has the potential to cause significant national socio-economic consequences through its impact on international trade, market disruptions involving two or more states and severe production losses to affected industries. EADRA Category 3 diseases have minimal or no effect on human health or the environment (AHA 2010; Department of Agriculture and Water Resources 2016).

4.9.2 Technical information

Pathogenesis

Two main forms of the disease are seen that relate to different pathogeneses. The first is localised disease where only localised lesions are seen, which normally represent 10–50% of cases in outbreaks (Hunter & Wallace 2001). This appears to be associated with intradermal inoculation of the virus. Affected animals do not normally develop demonstrable serological immunity, though they are generally resistant to subsequent viral challenge.

The second form of disease is seen where the virus directly enters the blood stream. It is believed to result from blood feeding insects causing intravenous inoculation. A severe systemic disease is seen with viraemia, and generalised clinical signs including inappetence, pyrexia, lachrymation, and reluctance to move followed by typical skin lesions and pox nodules in many organs about ten days later (House et al. 1990).

Clinical signs

Clinical signs of LSD may be acute, subacute or inapparent and are characterised by fever, and nodules within the skin which may be localised or generalised. Necrotic plaques are also often found in the mucous membranes in the mouth and upper respiratory tract and can cause a rapid

loss of condition and severe emaciation persisting for up to six months (Hunter & Wallace 2001). Peripheral lymph nodes may be swollen. Systemic clinical signs include pyrexia, anorexia, dysgalactia and pneumonia. The severity of the disease varies considerably between breeds and strains of cattle. Many cattle suffer severe emaciation and loss of production for several months. The skin lesions cause permanent damage to the hides (Davies 1991). The disease can also cause abortions in 1–7% of cows and infertility in bulls (Coetzer 2004).

Epidemiology

The incubation period for LSD virus is two to five weeks under field conditions (Weiss 1968) and five days under experimental conditions (Woods 1990). Viraemia usually lasts four to five days, and, under experimental conditions, up to 28 days. The virus may be present in saliva for 11 days post infection (Coetzer 2004; Weiss 1968). There is no evidence of a carrier state and naturally infected animals acquire lifelong immunity (Coetzer 2004).

All age groups appear to be equally susceptible to LSD virus. Experimental infection can affect giraffe and impala (Young, Basson & Weiss 1970) and antibodies to capripoxvirus have been detected in Cape buffalo, *Syncerus caffer*, in Kenya (Davies 1982) and in wildebeest, eland, springbok and impala in Republic of South Africa (Barnard 1997).

The mode of transmission has not been clearly established (Coetzer 2004). A few probings with a contaminated pin is sufficient to infect an animal (Callis 1996). Epidemiological investigations suggest that LSD virus transmission is usually mechanical by biting flies and mosquitoes, and on occasions by direct contact between animals (Davies 1991). Insects are not known to be maintenance hosts as the virus has not survived more than four days in insects (Weiss 1968). However, ixodid ticks were recently identified as having a role in the transmission of LSD with reports of transovarial transmission of LSD virus by *Rhipicephalus (Boophilus) decoloratus*, mechanical or intrastadial transmission by *Rhipicephalus appendiculatus* and *Amblyomma hebraeum* males, and trans-stadial transmission by *Amblyomma hebraeum* (Tuppurainen et al. 2015).

Intradermally feeding vectors are thought to be responsible for the enzootic status while intravenously feeding vectors such as mosquitoes are required to establish an epizootic. Carn and Kitching (1995) observed that generalised disease is more common after intravenous infection. Outbreaks are often associated with increased insect activity during wet summer months and in moist low-lying dairying districts with dense cattle populations (Weiss 1968).

LSD virus is readily transportable on fomites including clothing and equipment where it may persist for six months (AHA 2009). LSD virus is a stable virus, known to be very resistant in the environment (Coetzer 2004). Virus has been isolated from skin lesions until 39 days post infection, although viral nucleic material was detected in these lesions until 92 days post infection (Tuppurainen, Venter & Coetzer 2005). The virus is susceptible to sunlight, though it can survive in shaded animal pens for six months. It is also susceptible to detergents (AHA 2009).

Transmission has occurred via shared drinking troughs, probably due to contamination with infected saliva, and via infected milk to calves; but not via the conjunctival sac (Carn & Kitching 1995). There is no evidence of disease spread from handling infected animals (Coetzer 2004).

Live attenuated vaccines derived from the Neethling strain of LSD and sheep and goat pox viruses are available and effective in cattle vaccinated annually. Recombinant vaccines are being developed (OIE 2016c). However, these vaccines do not provide all animals with strong protection as some vaccinated animals can develop skin lesions containing high virus titres. Because of potential safety issues with the live attenuated LSD virus vaccine, its use is not recommended in countries free of the disease (Tuppurainen & Oura 2012).

Diagnosis

Laboratory confirmation of LSD virus is most rapid using a PCR method specific for capripoxviruses (Heine et al. 1999) or by the demonstration of typical capripox virions and intracytoplasmic inclusion bodies in biopsy material or desiccated crusts using the transmission electron microscope in combination with clinical history (Tuppurainen, Venter & Coetzer 2005).

Serological tests for LSD virus include indirect immunofluorescence, serum neutralisation, immunodiffusion tests and the antigen-detection ELISA, but these tests have either poor sensitivity or poor specificity (Gari et al. 2008; OIE 2016c).

4.9.3 Transmission in germplasm

Semen

LSD virus from infectious lesions on the scrotum, preputial mucosa and glans penis can contaminate semen. Nodules in testes can cause orchitis and render bulls infertile for 4–6 months or cause permanent infertility (Davies 1991).

LSD virus can also infect semen. Alexander and Weiss found LSD virus in semen of experimentally infected bulls for 22 days after fever and generalised skin lesions appeared (Weiss 1968). LSD virus was detected in semen of experimentally infected bulls for 42 days post infection by virus isolation and five months post infection by PCR, long after clinical signs had disappeared (Irons, Tuppurainen & Venter 2005; Osuagwuh et al. 2007). Virus was also detected for 28 days post infection in semen of experimentally infected unvaccinated bulls not showing clinical signs. LSD virus could not be detected in the semen of experimentally infected vaccinated bulls (Osuagwuh et al. 2007). The epididymis and testis were identified as the sites of persistence of LSD virus, and viral DNA was detected in all fractions of semen (Annandale et al. 2010).

Insemination of semen spiked with LSD virus under experimental conditions resulted in transmission to heifers (Annandale et al. 2014).

Embryo

LSD virus has caused multifocal necrotic lesions in the uterus and vagina of cows thus contaminating embryos with the virus (Annandale et al. 2014). Insemination of semen spiked with LSD virus under experimental conditions resulted in transmission to embryos; however, stepwise washing as per IETS Manual rendered the embryos free of LSD virus DNA (Annandale et al. 2014).

Currently the OIE Code Article 4.7.14 ranks LSD as an IETS Category 4 disease, that is, a disease for which studies have been done, or are in progress, that indicate: that no conclusions are yet possible with regard to the level of transmission risk; or the risk of transmission via in vivo derived embryo transfer might not be negligible even if the embryos are properly handled according to the IETS Manual between collection and transfer (OIE 2016f).

Collecting oocytes from clinically infected cows may not always be possible as a high percentage suffer from ovarian inactivity. The ovaries were smaller than average, and no activity was detected on the ovarian surface (Ahmed & Zaher 2008).

4.9.4 Current biosecurity measures

Australia has animal biosecurity measures for LSD virus for bovine semen and in vivo derived embryos from Canada and the United States.

The animal biosecurity measures require certification of country freedom from LSD as defined by the OIE Code (OIE 2016f).

4.9.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by the LSD virus:

- LSD is an OIE-listed disease and is nationally notifiable in Australia.
- LSD virus is not present in Australia, Canada and the United States.
- LSD mainly affects cattle, with occasional cases in Asian water buffalo (*Bubalis bubalis*).
- Clinical signs of LSD may be acute, subacute or inapparent and are characterised by fever and nodules in the skin which may be localised or generalised.
- There is evidence that LSD virus can be transmitted via bovine semen and there is a risk of transmission via bovine in vivo derived and in vitro produced embryos.
- Vaccination and serological tests of donors or donor herds do not provide adequate risk management against risk of entry, establishment and spread of LSD in Australia.

4.9.6 Conclusion

Based on the preceding factors, it was concluded that the overall risk of LSD virus infection associated with importing bovine germplasm has not changed, and is similar for bovine in vitro produced embryos. Hence animal biosecurity measures that applied to bovine semen and bovine in vivo derived embryos will also apply for bovine in vitro produced embryos from Canada and the United States.

The following biosecurity measures provide appropriate risk management.

For bovine in vitro produced embryos from Canada and the United States:

- At the time of, and for 30 days after, each oocyte collection for this consignment, Canada/the United States meets the OIE Code Article definitions for country freedom from lumpy skin disease.

4.10 Rift Valley fever

4.10.1 Background

Rift Valley fever (RVF) virus is a zoonotic, arthropod-borne virus that causes disease characterised by mortality in young domestic ruminants and abortions in pregnant animals. The virus can cause severe influenza-like disease in humans, with occasionally fatal complications (Swanepoel & Coetzer 2004; WHO 2010).

RVF virus can affect many species of animals including cattle, goats, sheep, buffalo, camels, monkeys and humans, as well as gray squirrels and other rodents. The primary amplifying hosts are cattle and sheep. Viraemia without disease may be seen in some adults of other species and severe disease can occur in newborn animals. Rabbits, pigs, guinea pigs, and chickens do not become viraemic (Swanepoel & Coetzer 2004). Animals do not develop carrier status with RVF (Swanepoel & Coetzer 2004).

RVF is endemic and widespread on the African continent, especially in sub-Saharan areas and Madagascar. Outside of Africa, outbreaks of RVF occur sporadically on the Arabian Peninsula (Arishi et al. 2000; Gould & Higgs 2009; Marley et al. 2008; OIE 2010; OIE 2016b). RVF has not been recorded in Australia, Canada and the United States; however, Australia has been shown to have competent mosquito vectors for RVF transmission (Turell & Kay 1998).

RVF is most commonly associated with mosquito-borne epizootics during periods of heavy rainfall and localised flooding. Major epizootics have occurred at irregular intervals of 5–20 years in southern and eastern Africa, causing heavy losses of animals and sometimes fatal human cases (Davies, Linthicum & James 1985; Swanepoel & Coetzer 2004). Generally, countries with any history of infection in live animals remain infected with RVF virus.

RVF is a multiple species OIE-listed disease (OIE 2016d). In Australia, it is nationally notifiable and is classed as an EADRA Category 2 disease, that is, a disease that has the potential to cause major national socio-economic consequences through very serious international trade losses, national market disruptions and very severe production losses in the livestock industries that are involved (AHA 2010; Department of Agriculture and Water Resources 2016).

4.10.2 Technical information

Pathogenesis

Following infection from bites of infected mosquitoes, RVF virus replicates initially in regional lymph nodes and is then distributed in blood plasma of infected hosts to target organs, particularly the spleen and liver. The virus replicates very quickly in these tissues and an intense viraemia develops. Within 12 hours of infection, detectable viraemia can occur in young animals with high titres for about five days (AHA 2016b), after which the level of infectivity falls rapidly as the level of antibodies rises (WHO 2010). RVF virus pathogenicity is believed to be related to a combination of viral-induced cell lysis in several organs and immunological reactions (Swanepoel & Coetzer 2004; Wood et al. 1990). In addition, the virus may localise in joints, spleen, liver, eye, central nervous system and reproductive tract, where it can be recovered for prolonged periods (AHA 2016b).

Up to 50% of sheep, including vaccinated sheep, can shed the virus through nasal, oral, rectal and vaginal routes for at least ten days after challenge (Saber et al. 1984).

Clinical Signs

The incubation period is 1–3 days in cattle and sheep. In newborn lambs, it is 12–36 hours. The clinical signs vary with the age, species and breed of the animal. In endemic regions, epidemics of RVF can be recognised by foetal malformations, high mortality rates in newborn animals and abortions in adults (Swanepoel & Coetzer 2004).

Newborn lambs are the most severely affected by RVF. Initial clinical signs include pyrexia, anorexia and lymphadenopathy. This is followed by weakness and death within 36 hours. Haemorrhagic diarrhoea or abdominal pain may also occur. In neonates, the mortality rate may reach 90–100%. In young calves similar clinical signs occur with mortality rates of 10–70%.

Abortions are the most characteristic clinical sign in adult cattle and sheep. Other clinical signs seen in adult sheep include pyrexia, weakness, nasal discharge, melena, diarrhoea and vomiting. Adult cattle may show pyrexia, anorexia, weakness, excess salivation, diarrhoea, decreased milk production and icterus. Milder but similar clinical signs occur in goats, whereas adult camels only show abortions. Abortion rates vary from 5% to almost 100% in ewes. Up to 85% of cattle have aborted in some outbreaks but the typical abortion rate is less than 10% (Swanepoel & Coetzer 2004).

Epidemiology

Adult mosquitoes infected with RVF virus usually remain so for life. At least one species can transmit RVF virus at 36 days after oral infection (Turell & Kay 1998). While environmental factors and availability of hosts govern the daily survival rate of the mosquito population, survival beyond four weeks is rare. Mechanical transmission by *Culex* species and other biting insects spreads RVF during epizootics. Transovarial transmission in *Aedes* species of mosquitoes, on the other hand, affects the persistence of RVF virus in the wild (Bath 2007; Swanepoel & Coetzer 2004). Non-vectorial transmission is important in humans but not in livestock. Slaughter of infected animals, necropsy procedures and laboratory manipulation of tissues and isolated viruses carry a high risk of disease transmission to humans (Swanepoel & Coetzer 2004).

Diagnosis

RVF can be diagnosed by isolation of the virus from the blood of pyrexemic animals and the liver, spleen and brain of dead animals and aborted fetuses (Geering, Forman & Nunn 1995c; van der Lugt, Coetzer & Smit 1996). The virus can be grown in numerous cell lines.

Viral titres in tissues are often high, allowing rapid diagnosis using complement fixation, virus neutralisation or agar gel diffusion tests on tissue suspensions. Viral antigens can be detected by immunofluorescent staining of impression smears from the liver, spleen or brain. Antigen-capture enzyme immunoassays and immunodiffusion tests can identify virus in the blood. Reverse transcription-polymerase chain reaction testing can detect viral RNA (Garcia et al. 2001; Sall et al. 1999).

Commonly used serological tests include virus neutralisation (the OIE prescribed test for international trade), enzyme-linked immunosorbent assays and haemagglutination inhibition tests (Paweska et al. 2005). Immunofluorescence, complement fixation, radioimmunoassay and immunodiffusion are used less frequently. Cross-reactions with other phleboviruses can occur in serological tests other than virus neutralisation.

4.10.3 Transmission in germplasm

Semen

It has not been demonstrated if infected donors can shed RVF virus in their semen (Radostits et al. 2007; Swanepoel & Coetzer 2004). However, RVF virus can be excreted in saliva, nasal, rectal and vaginal discharges, and possibly milk during the viraemic phase (Saber et al. 1984; Swanepoel & Coetzer 2004). Inflammatory cells and leukocytes which can be potentially infected with RVF virus may be secreted in seminal fluids for this period or longer. Thus it is highly probably the bovine semen can be infected with RVF virus during viraemia (Thibier and Guerin, 2000).

Embryo

Published research or reports on infection of in vivo derived or in vitro produced embryos with RVF virus are not available. Embryos collected from mice were found to be infected with RVF but this may have been due to blood contamination during surgical collection despite care taken (Marley et al. 2008; Mims 1956).

According to the OIE Code Article 4.7.14, the IETS has not yet ranked RVF in in vivo derived embryos. As RVF virus is known to infect several organs, it is highly probably the bovine embryos can be infected with RVF virus during viraemia (OIE 2016f).

4.10.4 Current biosecurity measures

Australia has animal biosecurity measures for RVF virus bovine semen and in vivo derived embryos from Canada and the United States.

The animal biosecurity measures require for certification of country freedom from RVF as defined by the OIE Code (OIE 2016f).

4.10.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by the LSD virus:

- RVF is an OIE-listed disease and is nationally notifiable in Australia.
- RVF virus is not present in Australia, Canada and the United States. Australia has been shown to have competent mosquito vectors for RVF transmission.
- The virus can affect many species of animals including cattle, goats, sheep and buffalo.
- The virus is a zoonotic, arthropod-borne virus that causes mortality in young domestic ruminants and abortions in pregnant animals.
- Major epizootics can occur, causing heavy losses of animals and sometimes fatal human cases. Countries with any history of infection in live animals remain infected with RVF virus.
- Should RVF enter, spread and establish in Australia, eradication is not likely to be possible because of the widespread presence of competent mosquito vectors.
- There is a risk of transmission via bovine semen and in vivo derived and in vitro produced embryos.

4.10.6 Conclusion

Based on the preceding factors, it was concluded that the overall risk of RVF virus infection associated with importing bovine germplasm has not changed, and is similar for bovine in vitro produced embryos. Hence animal biosecurity measures that applied to bovine semen and bovine in vivo derived embryos will also apply for bovine in vitro produced embryos from Canada and the United States.

The following biosecurity measures provide appropriate risk management.

For bovine in vitro produced embryos from Canada and the United States:

- Donors showed no clinical signs of disease within the period from 14 days prior to, and 14 days following oocyte collection.
- At the time of and for 30 days after each oocyte collection for this consignment, Canada/the United States meets the OIE Code Article definitions for country freedom from Rift Valley fever (RVF).

4.11 Vesicular stomatitis

4.11.1 Background

Vesicular stomatitis (VS) is an insect-transmitted viral disease that primarily affects horses, cattle, and pigs. VS is limited to the American continents although historically outbreaks were described in Europe in the early 1900s associated with the export of horses from the United States (OIE 2016c). It was last diagnosed in Canada in 1949 and has never been reported in Australia (OIE 2016b).

VS virus (VSV) is a single-stranded RNA virus in the genus *Vesiculovirus* of the family *Rhabdoviridae* (Tordo et al. 2005). Two serologically distinct serotypes exist, Indiana (IND) serotype (with three subtypes, IND-1, IND-2, IND-3) and New Jersey (NJ) serotype (OIE 2016c; Reis, Jr. et al. 2009).

The NJ and IND-1 serotypes are endemic in livestock in areas of southern Mexico, Central America, Venezuela, Colombia, Ecuador and Peru, with the NJ serotype causing the vast majority (>80%) of the clinical cases. Sporadic activity of NJ and IND-1 serotypes has been reported in northern Mexico and western United States. IND-2 has only been isolated in Argentina and Brazil and only from horses. The IND-3 subtype has been identified sporadically in Brazil where it is reported to cause disease more frequently in horses than cattle (Reis, Jr. et al. 2009).

VSV has a wide host range in animals, causing vesicular disease in equids (donkey, horse, mule), cattle and pigs. Goats and sheep are more resistant to clinical disease and are rarely affected (Reis, Jr. et al. 2009). Antibodies to VSV have been detected in a wide range of vertebrate species including primates (human and non-human), bovines, camelids, coyotes, foxes, dogs, hamsters, marsupials, rodents and birds (Jimenez et al. 1996; Johnson, Tesh & Peralta 1969). In addition, VSV has been isolated from many haematophagous and non-haematophagous insect species including sand flies, black flies, mosquitoes, culicoides, house flies, eye gnats and grasshoppers (Drolet, Stuart & Derner 2009; Rodriguez 2002). Definitive reports of VS in bison and buffalo are lacking but these species are listed as being susceptible to infection with VSV under state-administered animal health legislation within the United States.

VS is zoonotic and can cause an influenza-like illness in humans who have come into direct contact with infected livestock (Letchworth, Rodriguez & Barrera 1999; Reif et al. 1987).

VS is not an OIE-listed disease (OIE 2016d). In Australia, it is nationally notifiable and is classed as an EADRA Category 2 disease, that is, a disease that has the potential to cause major national socio-economic consequences through very serious international trade losses, national market disruptions and very severe production losses in the livestock industries that are involved (AHA 2010; Department of Agriculture and Water Resources 2016). It is significant in that it closely resembles FMD.

With respect to VS, Canada has import restrictions for bovine semen and embryos from the United States (premises free from VS for at least 30 days) (USDA 2015c; USDA 2015d). VS is reportable in Canada, in all states of the United States for all susceptible animals, and is included the 2016 U.S. National List of Reportable Animal Diseases (NLRAD).

4.11.2 Technical information

Pathogenesis

Knowledge concerning the natural transmission and pathogenesis of VSV remains incomplete (Reis, Jr. et al. 2009). It is generally assumed that animals acquire infection either through the bite of an infected competent insect vector, or exposure to a clinically affected host (McCluskey & Mumford 2000), or possibly ingestion of immature stages of grasshoppers infected with VSV (Drolet, Stuart & Derner 2009).

The course of disease depends on the site of inoculation. Clinical disease is more likely to occur when infected insects bite susceptible livestock in the mouth, nostrils or coronary band area (Mead et al. 2009; Scherer et al. 2007). By contrast, insect feeding (and viral inoculation) at the flank, ear and peri-ocular areas resulted in the development of low levels of neutralising antibody without the formation of vesicles (Mead et al. 2009). Infection of susceptible hosts appears to be enhanced by minor abrasions or trauma to skin or mucosal surfaces when compared to oral inoculation of unbroken surfaces (Howerth et al. 2006). Incubation period is 2–8 days.

The virus is epitheliotrophic being restricted in distribution to lesions of the skin, anterior alimentary tract mucosa and associated draining lymph nodes. In cattle, viraemia does not occur as a result of infection (Mead et al. 2009; Scherer et al. 2007).

Viral shedding from an active lesion appears to cease about 6–7 days after lesion formation (Katz et al. 1997). Persistent shedding of infective VSV from recovered animals is not known to occur (McCluskey & Mumford 2000).

Epidemiological data indicate that in cattle herds where the disease is endemic, up to 90% of animals may be seropositive with only 10% presenting typical clinical signs (Reis, Jr. et al. 2009).

Clinical signs

The incubation period is variable but vesicles are usually visible within 24–72 hours of virus inoculation (Reis, Jr. et al. 2009). Clinical signs of VS in cattle and pigs are mild pyrexia and the presence of vesicles that progress to erosions and ulcerations on the tongue, palate, gum, lips, snout (pigs), teats, prepuce, interdigital space and coronary band (Reis, Jr. et al. 2009). VS may be distinguished epidemiologically from FMD as the latter does not cause disease in horses (Reis, Jr. et al. 2009; Schmitt 2002).

Oral lesions cause animals to salivate excessively and to refuse feed resulting in weight loss; lameness may occur due to interdigital lesions (Bridges et al. 1997; Schmitt 2002). By the time affected animals are examined, vesicles have often ruptured and only erosive lesions or ulcers are present (McCluskey & Mumford 2000). VS is rarely fatal but mastitis, anorexia, dehydration and weight loss result in significant production losses in cattle (Bridges et al. 1997).

Epidemiology

The interplay of disease vectors and hosts in natural transmission of VS is not well understood. Morbidity rates vary widely between outbreaks and can be as high as 96% (Reis, Jr. et al. 2009). Disease spread tends to follow natural features such as valleys and rivers rather than predictable human or animal routes (Letchworth 1996). Experience in the United States is that, during outbreaks, a majority of VS positive premises are not contiguous with other VS positive premises (McCluskey, Hurd & Mumford 1999). Cattle and horses under one year of age are

rarely affected clinically. Mortality is close to zero in both cattle and horses, although high mortality rates have been observed in pigs affected by the NJ serotype (OIE 2016c).

Numerous insects have been implicated as both mechanical and biological vectors. These include sand flies, black flies and biting midges all of which are capable of transmitting the virus during blood feeding (Smith et al. 2012). A component of the saliva of some insects (e.g. black flies) may enhance VSV replication and transmission (Reis, Jr. et al. 2009). Migratory grasshoppers have been identified as efficient amplifying reservoir hosts (Drolet, Stuart & Derner 2009).

Pasture grasses can harbour viable VSV and grasshoppers fed on VSV-infected plant meal were found to harbour viable virus 21 days after feeding (Nunamaker et al. 2003). Grazing cattle consume significant numbers of grasshoppers during the insect's immobile moulting phases (Drolet, Stuart & Derner 2009), providing a plausible basis for a cattle-grasshopper-cattle transmission cycle. Migratory grasshoppers are known to travel greater than 45 km per day and geo-spatial correlations of VS outbreaks and grasshopper infestations have been observed (Nunamaker et al. 2003).

Vesicular fluids contain extremely high concentrations (in excess of 10^8 TCID₅₀/mL) of virus (Clarke, Stallknecht & Howerth 1996; Scherer et al. 2007) and prominent vesicular lesions are necessary for efficient animal-to-animal contact transmission (Reis, Jr. et al. 2009). Within herd spread is facilitated by direct contact with clinically affected animals and contact with fomites (e.g. feed, water troughs) contaminated by the virus being shed in saliva from oral lesions (Leder et al. 1983). Contamination of pasture grasses by VSV-infected saliva provides a plausible mechanism for transmission of infection to herbivorous insects in which virus amplification has been detected (Drolet, Stuart & Derner 2009).

Studies have also shown horizontal transmission of VSV between insects while co-feeding on (non-viraemic) mammalian hosts, theoretically making viraemia unnecessary for insect-to-insect transmission (Mead et al. 2000). Viraemia (after experimental infection) has only been reported in rodents, including laboratory mice, spiny rats, Syrian hamsters and deer mice and it has been suggested that deer mice and/or other native American rodents may be involved in the epidemiology of VS (Cornish et al. 2001).

Diagnosis

Laboratory diagnosis is crucial as VS is not easily distinguished from other vesicular diseases particularly FMD, vesicular exanthema and swine vesicular disease. However, the presence of symptoms in horses indicate VS.

In clinically affected livestock, VSV can be readily isolated and viral RNA can be detected from epithelial tissue and vesicular fluid by conventional and real-time reverse-transcriptase polymerase chain reaction (RT-PCR). The preferred immunological methods for identifying viral antigens are the enzyme-linked immunosorbent assay (ELISA), the complement fixation test (CFT) and fluorescent antibody staining. The virus neutralisation test is more time-consuming (OIE 2016c). For diagnostic specimens, real-time RT-PCR may be more sensitive than viral isolation or CFT (Letchworth 1996).

4.11.3 Transmission in germplasm

Semen

Published research or reports on infection of bovine semen with VSV are not available. However, there is a probability of extrinsic contamination of bovine semen, that is, vesicles rupturing and contaminating semen and/or equipment, in risk areas and for disease transmission to occur as a result of handling VSV contaminated material during artificial insemination.

Embryos

There is a risk of bovine embryos becoming contaminated with VSV from vesicles on the perineum. However, VSV cannot be completely removed by washing as per IETS Manual as the virus adheres to the bovine zona pellucida (Lauerman et al. 1986). Treatment with trypsin did not remove VSV from all washed ova (Stringfellow, Lauerman & Thomson 1989) but removed VSV from all washed embryos (Singh 1987; Singh & Thomas 1987).

There is a risk of zoonotic infection with VS as a result of using infected equipment. Although sunlight and disinfectants readily inactivate VSV, the procedures in embryo collection and processing are highly favourable for survival of the virus. Thus the virus is highly biohazardous and risk management measures are justifiable to ensure that the embryos, embryo straws, and the transport containers are not contaminated with VS when importing bovine embryos from VSV affected areas.

Consequently, the OIE Code Article 4.7.14 ranks VS in cattle and pigs as an IETS Category 4 disease, that is, a disease for which studies have been done, or are in progress, that indicate: that no conclusions are yet possible with regard to the level of transmission risk; or the risk of transmission via in vivo derived embryo transfer might not be negligible even if the embryos are properly handled according to the IETS Manual between collection and transfer (OIE 2016f).

4.11.4 Current biosecurity measures

Australia has animal biosecurity measures for VSV for bovine semen and in vivo derived embryos from Canada and the United States.

The animal biosecurity measures require for Canada to provide certification of country freedom from VS as previously defined by the OIE Code for both bovine semen and in vivo derived embryos. However, the OIE Code currently do not have any definition for country freedom from VS. For the United States, certification was more complex:

For bovine semen: VS was not reported within 15 kilometres of the Semen Collection Centre during the period 30 days before the first collection of semen for this consignment and until completion of the final collection of semen for this consignment.

For bovine in vivo derived embryo: VS was not reported within 80 kilometres of the premises where the male or female donors resided during the period from two months prior to the first collection for this consignment until one month after the final collection for this consignment.

4.11.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by the VSV:

- VS is not an OIE-listed disease but is nationally notifiable in Australia.

- VSV is limited to the American continent. It occurs in the United States. It was last diagnosed in Canada in 1949 and has never been reported in Australia.
- VS is reportable in Canada and some states of the United States.
- VS is a viral disease affecting horses, pigs and ruminants such as cattle and sheep.
- The disease is significant because it closely resembles foot and mouth disease (FMD). It causes a mild fever, and the formation of blister-like lesions on the inside of the mouth, and on the lips, nose, hooves and udder. The blisters break, leaving raw, sore areas. Affected animals often salivate profusely, and are unwilling to eat or drink.
- The epidemiology of VS, especially with respect to VSV transmission, is complex and not fully understood. However, VS can spread by direct contact. VS is notifiable in the United States and infected premises are quarantined. Quarantine and other biosecurity controls, e.g. fly control and county disease zoning, do reduce the risk of spread to areas outside endemic risk areas.
- Laboratory diagnosis is crucial to eliminate other vesicular diseases.
- There is a risk of transmission via contaminated bovine semen and in vivo derived and in vitro produced embryos.
- Canada has import restrictions for bovine semen and embryos from the United States (premises free from VS for at least 30 days) (USDA 2015c; USDA 2015d).

4.11.6 Conclusion

Based on the preceding factors, it was concluded that the overall risk of VS associated with importing bovine germplasm has not changed, and is similar for bovine in vitro produced embryos. Given that VS is no longer an OIE-listed disease and therefore the OIE no longer recognises country freedom from VS, amended animal biosecurity measures are warranted for bovine in vitro produced embryos from Canada and the United States. Given the high volume of trade between Canada and the United States and the higher risk of exposure to VS in Canada, the recommended biosecurity measures are similar to the Canadian biosecurity measures.

The following biosecurity measures provide appropriate risk management.

For bovine in vitro produced embryos from Canada and the United States:

- During the 30 days prior to, and at the time of, each collection of oocytes, there was no clinical signs or reports of vesicular stomatitis in premises where donor cows were kept and at the oocyte collection facility.

4.12 Brucellosis

4.12.1 Background

Brucellosis in cattle is caused by *Brucella abortus*, occasionally by *B. melitensis* and rarely by *B. suis*. It is a highly contagious disease characterised by abortion and infertility in cows and undulant fever in humans.

B. abortus infects mainly cattle but can also infect sheep, goats, pigs, horses and other livestock and free-living species, such as bison, cervids, and opossums, as well as humans. However, infection does not generally spread in non-bovid species. Eight biovars of *B. abortus* (1, 2, 3, 4, 5, 6, 7 and 9), divided on the basis of cultural and serological properties have been identified (Godfroid et al. 2004). Some biovars may have distinct epidemiologic features (Godfroid et al. 2005).

Bovine brucellosis due to *B. abortus* is found in most countries except where eradicated as a result of national regulatory programs. Australia has been free from bovine brucellosis due to *B. abortus* since 1989. *B. suis* is present in Australia and has been detected in cattle (Cook & Noble 1984). *B. melitensis* is not present in Australia.

Canadian livestock has been free from brucellosis since 1986, but *B. abortus* remains endemic in wood bison in northern Alberta, specifically in Wood Buffalo National Park where the bacterium has also been found in wolves, fox and moose. *B. suis* biovar 4 has been reported from barren-ground caribou and reindeer and is endemic in Manitoba. *B. melitensis* is not reported in Canada.

All states of the United States are classed by the USDA as free from *B. abortus* in cattle. However, the infection remains in wildlife, particularly elk (*Cervus elaphus*) and sometimes bison, in and around the Yellowstone area, with occasional spread to cattle. Since 2007, brucellosis was detected in cattle in the states of Montana, Wyoming and Idaho (USDA 2015a). *B. suis* is reported in pigs and cattle in the United States (Ewalt et al. 1997). *B. melitensis* is not reported in the United States.

B. abortus, *B. melitensis* and *B. suis* are OIE-listed diseases (OIE 2016d). In Australia, they are nationally notifiable and the first two (*B. abortus*, *B. melitensis*) are classed as an EADRA Category 2 disease, that is, a disease that has the potential to cause major national socio-economic consequences through very serious international trade losses, national market disruptions and very severe production losses in the livestock industries that are involved (AHA 2010; Department of Agriculture and Water Resources 2016).

All three species are zoonotic, the most pathogenic and invasive species for humans being *B. melitensis* (Seleem et al. 2010).

4.12.2 Technical information

Pathogenesis

Infection with *Brucella* spp. depends mainly on strain, virulence of the bacteria, infective dose, and immunity, age, sex and reproductive status of the host animal (Godfroid et al. 2004).

Infection starts when *Brucella* spp. penetrate the mucosa or skin and are ingested by neutrophils and macrophages, which then transport the bacteria to the draining lymph nodes where they might multiply. Further spread via blood to other lymph nodes and the reticuloendothelial cells often follows.

Bacteraemia might last for several months, resolve or, in a small proportion of animals, recur. During bacteraemia, the bacteria are carried within neutrophils and macrophages or transported free in the plasma to various organs, particularly the endometrium of the gravid uterus, udder and supramammary lymph nodes, and, if pregnant, the foetal membranes. Localisation might also occur in the spleen and synovial structures. In bulls, the bacteria might localise in the testes and male sex glands (Adams 2002).

Disease expression depends on the intracellular survival and persistence of *Brucella* spp. within these phagocytic cells in the various parts of the animal and the extent of injury caused by the organism.

Clinical signs

B. abortus infection causes mid-term to late-term abortions and infertility in cows and less commonly orchitis in bulls with purulent arthritis, bursitis or tendovaginitis in both cows and bulls (Godfroid et al. 2004). Bacteraemia usually resolves after several months but can recur for up to two years, particularly in calving cows. The bacteria usually localise in regional lymph nodes and in the uterus and udder of cows; or testes, epididymis and sex glands of bulls.

B. melitensis or *B. suis* infection in cattle is often asymptomatic, but is excreted in milk (Alvarez et al. 2011; Ewalt et al. 1997)

Epidemiology

Transmission of *B. abortus* is usually by ingestion of infective material such as the placenta of an aborted foetus or licking the genitalia of infected cows. Calves can acquire infection in utero or by ingesting infected colostrum or milk. Latent infection of heifers born to seropositive cows may not be evident serologically until their first gestation, when abortion may occur (Godfroid et al. 2004).

Cattle can become naturally infected with *B. suis* following contact with infected pigs but the pathogen is not contagious within cattle population, even from cow to calf despite the pathogen being excreted in milk (Ewalt et al. 1997). Cattle mixed with small ruminants infected with *B. melitensis* may also become infected and calves drinking milk from infected dams may become infected (Alvarez et al. 2011).

Vaccination is highly effective in reducing the prevalence of bovine brucellosis and has contributed to the success of many control programs. Strains S19 and RB51 are the two *B. abortus* vaccines commonly used, being effective in the prevention of abortion and infection, and providing long lasting protection. Vaccination with S19 is now uncommon because of the risks of orchitis and shedding of the vaccine virus in semen. In addition, cattle vaccinated with strain 19 as calves will usually be positive to buffered antigen tests. Vaccination with strain RB51 is preferred as the vaccine organism is not associated with shedding or colonisation in tissues or semen (Edmonds et al. 1999). Animals vaccinated with RB51 are also likely to be negative to both buffered antigen and CFT tests (Radostits et al. 2007). The withholding period for RB51 is three weeks; however, Cheville and others (1996) recovered the RB51 strain from the superficial cervical lymph node 14 weeks after vaccination. Olsen and others (1999) observed the RB51 strain persisting in some vaccinated cattle to adulthood but could not determine its incidence or significance.

Diagnosis

Diagnosis is based on isolation and identification of *Brucella* organisms and on serological tests which detect antibodies in blood, milk, whey, vaginal mucous or seminal plasma. However, the results of serological tests need to be carefully interpreted against OIE recommendations and the bovine brucellosis control and vaccination program of each country (OIE 2016c).

4.12.3 Transmission in germplasm

Semen

It was once suspected that bulls were important in the transmission of brucellosis (Schroeder & Cotton 1916), but natural service is not an important method of spread (King & Kinross 1940). *B. abortus* can cause orchitis, epididymitis and seminal vesiculitis in bulls and be found in the necrotic and desquamating seminal epithelial cells and in macrophages shed in semen, seminal fluid and urine (Bendixen & Blom 1947; Robison et al. 1998). Infected bulls usually excrete the organism in the semen during the acute stage but as the disease becomes chronic, excretion may cease or become intermittent. *B. abortus* was isolated from 80 consecutive ejaculates collected from a bull over an 18 month period (Manthei, DeTray & Goode, Jr. 1951).

B. melitensis in the seminal fluid fraction of semen of bulls and rams have been identified by both PCR and direct culture method (Amin et al. 2001).

There is no report of *B. suis* in semen of bulls.

Embryo

In a review of research on embryo transfer from *B. abortus* infected cows, it was concluded that in vivo derived embryos are unlikely to be exposed to *B. abortus*, even in infected embryo donors. Furthermore, *B. abortus* can be effectively washed from in vivo derived bovine embryos with intact zona pellucida and exposed in vitro to *B. abortus*, but not from those with defective zona pellucida (Stringfellow & Wright 1989).

Consequently, the OIE Code Article 4.7.14 ranks *B. abortus* in cattle as an IETS Category 1 disease, that is, a disease for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the bovine in vivo derived embryos are properly handled (that is, no embryos with defective zona pellucida) and washed between collection and transfer (OIE 2016f).

With regards to in vitro produced embryos, *B. abortus* could not be detected in any sample of the recovery medium or in any group of ova collected non-surgically from super-ovulated cows artificially infected with *B. abortus*, washed ten times and then cultured for the isolation of *B. abortus*. Although the bacterium is a facultative intracellular coccobacilli capable of invading a range of phagocytic and non-phagocytic host cells, especially the trophoblasts in the uterus, it does not appear to invade the oocytes (Stringfellow, Panangala & Galik 1988). However, there is a risk of contamination of oocytes when collecting using the ovum pickup technique through the uterine wall of an infected cow.

Published research or reports of *B. melitensis* or *B. suis* in bovine embryos are not available.

4.12.4 Current biosecurity measures

Australia has animal biosecurity measures for bovine brucellosis (*B. abortus*) for bovine semen from the United States but no animal biosecurity measures for bovine in vivo derived embryos from either Canada or the United States.

The animal biosecurity measures for semen from the United States requires certification of freedom from bovine brucellosis (*B. abortus*) as previously defined by the OIE Code, or as a herd certified free for 5 years, or from a Class Free (brucellosis) State or area (OIE 2016f).

4.12.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by *B. abortus*:

- Brucellosis is an OIE-listed disease and bovine brucellosis (*B. abortus*) is nationally notifiable in Australia.
- *B. abortus* is not present in Australia but is present in parts of Canada and the United States populated with susceptible wildlife.
- Bovine brucellosis is not primarily a sexually transmissible disease but the bacteria can be present in semen of infected bulls.
- The bacteria are not associated with bovine oocytes and embryos, but, if present as a contaminant, the in vivo derived embryos can be treated by washing according to the procedures described in the IETS Manual. There is no treatment available for in vitro produced embryos.
- Neither vaccination of donors and donor herds nor diagnostic testing of donors nor donor herds not recognised free from bovine brucellosis provide adequate risk management against risk of entry, establishment and spread of bovine brucellosis in Australia.

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by *B. melitensis*:

Brucellosis due to *B. melitensis* is an OIE-listed disease and is nationally notifiable in Australia.

- *B. melitensis* is not present in Australia, Canada and the United States.
- There is no information on the risk of transmission via bovine semen or embryos although the bacteria has been isolated from bovine semen.
- *B. melitensis* is regarded as the most pathogenic and invasive of all *Brucella* species in humans.

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by *B. suis*:

- Brucellosis due to *B. suis* is an OIE-listed disease and is nationally notifiable in Australia.
- *B. suis* is present in Australia, Canada and the United States.
- *B. suis* is not contagious within cattle population.

4.12.6 Conclusion

Based on the preceding factors, it was concluded that the overall risk of bovine brucellosis due to *B. abortus* associated with importing bovine germplasm has not changed and animal biosecurity measures that applied to bovine semen will also apply for for bovine in vitro produced embryos.

The following biosecurity measures provide appropriate risk management.

For bovine in vitro produced embryos from Canada:

- During the 30 days prior to, and at the time of, each collection of oocytes, the donor cows were from a herd that was located in a country or zone free from infection with bovine brucellosis (*B. abortus*) and the herd was certified free without vaccination by CFIA in accordance with the current OIE Code.

For bovine in vitro produced embryos from the United States:

- During the 30 days prior to, and at the time of, each collection of oocytes, the donor cows were from a herd that was located in a country or zone free from infection with bovine brucellosis (*B. abortus*) in accordance with the OIE Code.
(Note: this includes donor cows from a herd that was located in a State or area officially free from infection with bovine brucellosis (*B. abortus*) and the herd was recognized as officially free of brucellosis by USDA-APHIS in accordance with the USDA Brucellosis Eradication Program.)
- Embryos derived from oocytes collected within six months of vaccination of donors against brucellosis are ineligible for export to Australia.

It was also concluded that animal biosecurity measures for brucellosis due to *B. melitensis* are required for bovine in vitro produced embryos.

- At the time of, and for 30 days after, each oocyte collection for this consignment, Canada/the United States meets the OIE Code Article definitions for country freedom from brucellosis due to *B. melitensis*.

Based on the preceding factors for *B. suis*, it was concluded that risk management measures are not warranted for *B. suis* in bovine in vitro produced embryos from Canada and the United States.

4.13 Paratuberculosis

4.13.1 Background

Paratuberculosis or Johne's disease (JD) is a chronic infectious enteritis of animals caused by the bacterium *Mycobacterium avium* subsp. *paratuberculosis* (*Map*). Nucleic acid detection techniques have detected distinct cattle and sheep strains (Collins et al. 1993), though cross infection has occurred (Whittington et al. 2001). An intermediate pattern has been identified in bacteria from sheep in the Republic of South Africa and Canada (de Lisle et al. 1992).

JD is recognised worldwide but, because it is a difficult disease to diagnose and the causative organism difficult to culture, its actual distribution cannot be accurately determined. JD is present in the United States and Canada.

Map can infect several different animal species including primates, but is particularly prevalent in dairy herds and is an emerging disease in other domestic livestock such as sheep, goats, camelids and farmed deer (Buergelt, Bastianello & Michel 2004). The organism has also been isolated in Scotland in wild rabbits and their predators—foxes, weasels and stoats (Beard et al. 2001).

Paratuberculosis is a multiple species OIE-listed disease (OIE 2016d).

Australia has relatively little JD compared to most developed agricultural countries, with large areas disease free (AHA 2015a). JD is nationally notifiable (Department of Agriculture and Water Resources 2016).

4.13.2 Technical information

Pathogenesis

After ingestion, *Map* localises in the mucosa of the small intestine and associated lymph nodes and to a lesser extent in the tonsils and retropharyngeal lymph nodes. Bacteria multiply primarily in macrophages of the lamina propria and submucosa of the terminal small intestine and large intestine, resulting in chronic diarrhoea and malabsorption and leakage of protein into the gastrointestinal tract resulting in muscle wasting, hypoproteinaemia and oedema.

Dissemination occurs when bacteria are carried by macrophages to other tissues, e.g. uterus, foetus, mammary gland, testes, liver, kidneys and lungs (Buergelt, Bastianello & Michel 2004). Microgranulomas caused by *Map* have been described in other lymph nodes and organs in mature cattle (Radostits et al. 2007).

Cattle are usually exposed to *Map* within the first few months of life and cattle older than ten months are relatively resistant to infection. Immunity is initially cell-mediated but this wanes as disease progresses from subclinical to clinical. A humoral response to infection develops late in the course of disease and therefore does not provide protection. In the late stages of disease, anergy might occur and neither cell-mediated nor humoral immune responses might be detectable (Radostits et al. 2007).

Clinical signs

Animals infected with *Map* might be in one of three groups, depending on whether they develop resistance after infection. The first group are infected but do not show clinical signs or shed bacteria in faeces. The second group do not show clinical signs but shed bacteria (carrier adult

cattle) and the third group show clinical signs and shed bacteria intermittently or continuously (Radostits et al. 2007).

The period between infection and the onset of clinical signs in naturally infected animals is prolonged, with clinical disease most common in cattle and sheep over two years old. High infective doses under experimental conditions can lead to a shorter incubation period and clinical signs within a year (Whittington & Sergeant 2001).

Clinical disease is characterised by a progressive weight loss leading to emaciation, oedema and poor coat quality. Frequently, chronic intractable diarrhoea occurs. Milk yield might drop by up to 20% in infected herds of dairy cattle and the herd reproduction rate is reduced. Death results from emaciation and chronic diarrhoea (Buergelt, Bastianello & Michel 2004).

Epidemiology

Transmission is mainly by the faeco-oral route, particularly during the post-natal period, and occasionally by direct (including pre-natal transmission) or indirect contact between animals (Sweeney, Whitlock & Rosenberger 1992; Whittington & Windsor 2009).

The lowest infectious oral dose of *Map* in experimental infection of cattle was 10^3 bacteria but typically 10^9 – 10^{12} bacteria were administered, often repeatedly. However, naturally infected animals might become infected at lower doses with a corresponding increase in the time for lesions and clinical disease to develop (Begg & Whittington 2008; Sweeney 1996).

Most animals become infected by sucking infected dams or from grazing contaminated pastures, soil, water or feed (Sweeney 1996). Epidemiological and experimental studies show that young animals are more susceptible to infection than older animals (Windsor & Whittington 2010). Older animals require unnaturally high doses of *Map* for infection to occur (Whittington & Sergeant 2001).

Infected animals can excrete *Map* in faeces before clinical signs are evident and sometimes in colostrum, milk, uterine fluids and semen (Buergelt, Bastianello & Michel 2004). They can continue to shed the bacteria continuously or intermittently for the rest of their lives (Whittington & Sergeant 2001). Faecal shedding starts at a younger age in herds with high rates of infection. In dairy herds with a prevalence of *Map* greater than 20%, about 20% of cattle less than two years old were positive on faecal culture (Weber et al. 2010).

Map organisms might also spread on farms in dust particles by aerosol (Eisenberg et al. 2010).

Live attenuated and killed vaccines are available as an aid to prevent clinical disease rather than preventing infection. The reduction in excretion rate is generally used as a measure of vaccine efficacy. Current vaccines have the disadvantage that they interfere with serological tests for *Map* and the delayed-type hypersensitivity test for bovine tuberculosis. Only faecal tests can be used for diagnosis of infection in individual vaccinated animals (OIE 2016c).

Diagnosis

Detection of *Map* infection in animals without clinical signs is limited by poor test sensitivity and specificity (Nielsen & Toft 2008). Histopathology of intestinal tissues and culture of intestinal tissues and faeces are the most sensitive tests.

The most sensitive and specific test for serum antibodies to *Map* is Ab-enzyme-linked immunosorbent assay (Ab-ELISA). The sensitivity of the Ab-ELISA is about 50% in adult subclinically infected cattle, about 15% in low shedder cattle and about 30% in low prevalence herds. The agar gel immunodiffusion test has a low sensitivity (10–30%) in cattle and goats but in sheep has a sensitivity of 78–93% and a specificity of 98–100% (Cousins et al. 2002).

Bacteriological culture of faeces is the most sensitive herd level test (Whittington & Sergeant 2001). PCR assays for *Map* in tissues and faeces are less sensitive than culture (Cousins et al. 2002). However, real time PCR assays have been used to detect *Map* in the tissues of slaughter cattle (Bosshard, Stephen & Tasara 2006) and in carcasses contaminated with faeces. Tests to detect paratuberculosis in cattle have not been validated for North American bison (Buergelt et al. 2000), although PCR tests on intestinal tissues and mesenteric lymph nodes detected all of 25 free ranging bison considered to have been infected with *Map* (Ellingson et al. 2005). In water buffalo PCR assays of intestinal tissue and mesenteric lymph nodes have been used (Sivakumar, Tripathi & Singh 2005).

4.13.3 Transmission in germplasm

Semen

Map has been recovered from the testes, prostate, bulbourethral gland, seminal vesicles and semen of infected bulls (Jorge et al. 1998; Thoen et al. 1977). Excretion of the bacteria in semen appears to be intermittent with only one to three colonies per mL of semen of bulls with clinical paratuberculosis cultured (Larsen et al. 1981), but whether semen can transmit the disease via uterus has not been investigated.

Embryo

Map can be cultured from the uterine flush of a cow with clinical paratuberculosis (Rohde et al. 1990). COCs and follicular fluids from infected cows have tested positive for *Map* (Bielanski et al. 2006) but washing in vivo derived and in vitro produced embryos according to the IETS Manual did not result in either transmission of disease to recipients or positive tests for *Map* (Bielanski et al. 2006; Kruip et al. 2003; Perry et al. 2006).

Currently, for bovine in vivo derived embryos, the OIE Code Article 4.7.14 ranks *Map* as an IETS Category 3 disease, that is, a disease for which 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 (OIE 2016f).

4.13.4 Current biosecurity measures

Australia has animal biosecurity measures only for *Map* for bovine semen from the United States. The animal biosecurity measures require for certification that each donor showed no clinical signs of JD during the semen collection period.

4.13.5 Risk review

The following key points were drawn from the preceding information to inform the review of the biosecurity risk presented by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*):

- JD is an OIE-listed disease and nationally notifiable in Australia.

- *Map* occurs world-wide and JD is present in Canada, the United States and in parts of Australia.
- *Map* infects several different animal species, especially dairy herds. It is an emerging disease in other domestic livestock such as sheep, goats, camelids and farmed deer.
- Transmission is mainly in faeces to young animals by infected adults. Older animals require extremely high doses of *Map* for infection to occur.
- Clinical disease is characterised by a progressive weight loss leading to emaciation, oedema and poor coat quality. Frequently, chronic intractable diarrhoea also occurs.
- Vaccination and serological tests of donors or donor herds do not provide adequate risk management against risk of entry, establishment and spread of *Map* in Australia.
- There is a risk of infection of bovine semen and pre-washed in vivo derived and in vitro produced embryos. However, there is no evidence of transmission via semen or embryos. Washing of embryos according to IETS Manual recommendations most likely reduces bacterial numbers to very low levels. High doses of *Map* are required to infect adult animals and the low dose of *Map* in semen and embryos is too low for infection in recipients.

4.13.6 Conclusion

Based on the preceding factors, it was concluded that there is a negligible risk of transmission of *Map* via bovine germplasm. Consequently, animal biosecurity measures for bovine in vitro produced embryos will not be required.

4.14 Bovine genital campylobacteriosis

4.14.1 Background

Bovine genital campylobacteriosis (BGC) is a venereally transmitted bacterial disease characterised by temporary infertility of female cattle. Abortion occurs in a small percentage of infected cows, months after initial infection. Infected bulls show no clinical signs but many become carriers and subsequently infect females at service. All breeds of cattle are susceptible.

BGC is caused by *Campylobacter fetus* subsp. *venerealis* and is regarded to have a world-wide distribution. It is present in Australia, Canada and the United States (Hoffer 1981; Waldner et al. 2013) but has not been isolated in New Zealand since 1993 (Sansone 2005).

BGC is an OIE-listed disease (OIE 2016d) but is not nationally notifiable in Australia (Department of Agriculture and Water Resources 2016). It is a significant reproduction disease typically not found on farms having good biosecurity practices. In support, many countries require certification of negative disease status.

4.14.2 Technical information

Pathogenesis

C. fetus subsp. *venerealis* is a venereally transmitted obligate parasite of cattle, adapted to the genital mucosa lining the preputial and vaginal cavities.

The bacteria is usually introduced into the cervico-vaginal area at oestrus by an infected bull and do not become established in the uterus until the progestational phase, when fewer neutrophils are present. In bulls, the bacteria usually concentrate in lumina of the epithelial crypts in the fornix of the prepuce and on the penis (Hoffer 1981).

Clinical signs

The disease is as a result of a subacute diffuse mucopurulent cervicitis, endometritis and salpingitis. BGC is characterised by temporary infertility of female cattle, that is, by late return to oestrus or by irregular extended oestrus cycles. In a small percentage of infected animals, sporadic abortion may occur, usually around the third trimester of pregnancy. Otherwise, no obvious clinical signs can be detected (Truysers et al. 2014).

Epidemiology

The infection is commonly introduced to a herd by a persistently infected bull, and less commonly by a persistently infected female. Bulls become infected when mating with an infected cow and transmit the bacteria from one female to another. Unless semen is appropriately treated with antibiotics, the disease can be spread by artificial insemination (Hoerlein et al. 1964; Shin et al. 1988). Direct female to female spread is unlikely; however, bull to bull transmission has been suspected among bulls penned together where riding behaviour is active.

Most infected heifers rid themselves of the organism within six months of sexual rest. Consequently, the problem does not become apparent until at pregnancy evaluation or at calving time, when only a limited number of females may still be infected and antibodies may have disappeared. Bulls are usually persistently infected but some can be successfully treated with vaccination and antibiotics, and immunity maintained with regular vaccination.

The disease is controllable and can be eradicated by vaccinating all breeding cattle (Sansone 2005). Other options include segregating potentially infected and uninfected animals in combination with extensive culling but this requires meticulous records and strict adherence to herd biosecurity practices (Truylers et al. 2014).

Diagnosis

BGC is usually diagnosed on the basis of herd history and laboratory investigation of vaginal or preputial swabs.

For artificial insemination, preputial samples or semen taken from bulls are analysed for the presence of the causal organism. Confirmation of the isolate can be performed by biochemical or molecular methods. Screening of mature bulls prior to entering semen collection centres usually require serial testing to ensure high sensitivity (Meyer 2014).

The ELISA can be used for testing herd immunity, but is not suited for evaluating infection in individual animals (Sansone 2005).

4.14.3 Transmission in germplasm

Semen

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; van Bergen et al. 2006).

Embryos

Published research or reports of *C. fetus* subsp. *venerealis* transmission via bovine in vivo derived embryos are not available. The bacteria infect the uterus and cause early embryonic and foetal mortalities, and infertility, affecting embryo production. It is common practice to add antibiotics to collecting fluids and wash fluids.

Insemination of oocytes with semen contaminated with *C. fetus* subsp. *venerealis* resulted in lower rates of fertilisation and development of in vitro produced embryos (Bielanski et al. 1994). Washing procedures and antibiotic treatment for the removal of *C. fetus* subsp. *venerealis* from in vitro produced embryos have not been completely effective but it was observed that in vitro produced embryos contaminated with *C. fetus* subsp. *venerealis* were rendered free of the infectious agent after washing according to the IETS Manual procedures (Bielanski et al. 1994).

Countries that are major producers and importers of bovine embryos generally do not require biosecurity measures for either in vivo derived or in vitro produced embryos.

4.14.4 Current biosecurity measures

Australia has animal biosecurity measures for *C. fetus* subsp. *venerealis* for bovine semen from Canada and the United States but not for bovine in vivo derived embryos.

4.14.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by *C. fetus* subsp. *venerealis*:

- BGC is an OIE-listed disease but is not nationally notifiable in Australia.

- BGC is a venereal disease of cattle and *C. fetus* subsp. *venerealis* is transmissible by artificial insemination.
- Infection cause early embryonic deaths, abortions and infertility in female cattle but does not cause any clinical signs in bulls.
- Cows recover from infection within a few months but bulls, unless successfully treated by vaccination and antibiotics, are usually persistently infected.
- Appropriate antibiotics added to flushing fluids, washing fluids and culture fluids help to remove some bacteria infecting embryos.
- *C. fetus* subsp. *venerealis* does not survive washing after in vitro culture.

4.14.6 Conclusion

Based on the preceding factors, it was concluded that animal biosecurity measures are not warranted to manage the risk of transmission of *C. fetus* subsp. *venerealis* in bovine in vitro produced embryos from Canada and the United States.

4.15 Bovine tuberculosis

4.15.1 Background

Bovine tuberculosis (TB) is an infectious disease affecting mainly cattle but which can be transmitted to all warm-blooded vertebrates, including humans. It is characterised by formation of nodular granulomas or tubercles in various organs throughout the body. Infection, caused by *Mycobacterium bovis*, an acid-fast bacterium, is usually life-long with gradual loss of condition and a cough. Treatment in cattle is usually uneconomic, unrewarding and rarely attempted.

Cattle are the main source of bovine TB; however, several wildlife species and feral animals also act as maintenance hosts for infection and reservoirs of infection for cattle. Examples include badgers in the United Kingdom, brushtail possums in New Zealand, and wild deer, elk and bison in North America (USDA 2015b).

Spoligotyping of *M. bovis* has identified two groups of strains of *M. bovis*, the bovine group and caprine group. The bovine group infects mainly cattle, deer, pigs and wild animals while the caprine group infects mainly sheep and goats (Aranaz et al. 1996). Only the bovine group will be assessed here.

Bovine TB occurs worldwide although it is now rare in countries that have introduced strict control and eradication measures.

Australia is officially free from bovine TB as a result of a successful national eradication program. No cases have been detected since 2002.

Although bovine TB was last reported in domestic livestock in Canada in 2011, it is present in wild deer and elk, especially around the Riding Mountain region in southern Manitoba. It is also present in the United States at very low levels in domestic herds with only two herds infected in 2014, down from 13 herds in 2010 (USDA 2015a). States, except California and part of Michigan, are classed as Accredited Free, that is, having zero per cent prevalence of affected cattle and bison herds, and no findings of tuberculosis for the previous five years, with some exceptions provided for in the Code of Federal Regulations (9 CFR 77.5) (USDA 2015b).

Bovine TB is an OIE-listed disease (OIE 2016d). In Australia, it is nationally notifiable and is classed as an EADRA Category 4 disease, that is, a disease classified as being mainly a production loss disease. While there may be international trade losses and local market disruptions, these would not be of a magnitude that would be expected to significantly affect the national economy. The main beneficiaries of a successful emergency response to an outbreak of bovine tuberculosis would be the affected livestock industry (AHA 2010; Department of Agriculture and Water Resources 2016).

4.15.2 Technical information

Pathogenesis

M. bovis spreads within the body in two stages. The first stage is the formation of primary lesions or tubercles at or near the point of entry or local lymph node as early as 20 days post infection. Infection via inhalation often results in infectious lesions in the cranio-ventral lungs or their regional lymph nodes. The second stage is the dissemination from the primary lesions and the formation of multiple discrete nodules in other organs, sometimes not involving local lymph nodes (Radostits et al. 2007). Sometimes lesions form in testes, uterus and ovaries (OIE 2016c).

Immunity is cell-mediated and therefore cannot be transferred e.g. calves cannot receive any maternal immunity from their mother's milk. The cell-mediated immune response provides not only protective responses against the bacteria but also contributes to the formation of characteristic granulomatous lesions (Neill et al. 1994).

There is a humoral response to infection but it occurs late in the course of disease and therefore does not provide protection.

Clinical signs

Infection in cattle is characterised by a long incubation period with clinical signs taking up to seven years to develop. Clinical signs in tuberculosis are rare and generally depend on the route of infection. Consumption of infected milk, contaminated feed and water or swallowing infected cough phlegm might produce lesions in the digestive tract and associated lymph nodes, including those of the oropharynx. This has led to swallowing difficulties, abdominal pain and ascites (Addison 1983). Infection via aerosols produces lesions in the lung and associated lymph nodes and this might eventually lead to coughing. More rarely, cutaneous infection introduced by trauma has caused localised infection, occasionally involving associated lymph nodes. This could lead to skin lesions being observed.

Where infection with *M. bovis* is generalised and progressive in cattle, goats, sheep and horses, a characteristic productive chronic cough indicating extensive pulmonary involvement sometimes develops after several months or even years. Cattle could also develop progressive emaciation accompanied by capricious appetite, fluctuating temperatures and weakness.

Epidemiology

Transmission is mainly by inhalation of infected aerosols from a coughing or sneezing tuberculous animal. Alimentary infection may occur in calves drinking milk of cows with tuberculous mastitis, or in cattle eating contaminated feed and water. Transmission via the congenital, cutaneous, or venereal route has been reported. The bacteria may be excreted in respiratory discharges, sputum and saliva, faeces, milk, urine, vaginal and uterine discharges as well as discharges from open peripheral lymph nodes. Infected cattle not showing clinical signs can excrete bacteria. Animals with gross tuberculous lesions communicating with airways or the intestinal tract are likely to excrete large numbers of bacteria into the environment (Cousins et al. 2004; Radostits et al. 2007).

Vaccines are being developed and tested, but currently are not commercially available.

Diagnosis

The delayed-type hypersensitivity test is the standard test for detecting TB. It involves injecting bovine tuberculin intradermally and measuring the subsequent swelling at the site of injection three days later. Response to the test depends on the immune response of the animal to infection. New diagnostic blood tests are available, e.g. the lymphocyte proliferation assay, the gamma-interferon assay, and ELISA. Due to the cost and more complex nature of laboratory-based assays, they are usually used as ancillary tests to confirm or negate the results of a positive intra-dermal skin test (OIE 2016c).

4.15.3 Transmission in germplasm

Semen

M. bovis can infect semen intrinsically and extrinsically. Intrinsic infection occurs when mobile phagocytes containing viable *M. bovis* facilitate the passage of the bacteria into semen (Niyaz Ahmed, Khan & Ganai 1999). Although uncommon, miliary tuberculosis and chronic testicular tuberculosis have been reported in testes of bulls (Hein & Tomasovic 1981). Extrinsic infection occurs in bulls with TB lesions in the prepuce.

Embryos

Published research or reports on infection of in vivo derived or in vitro produced embryos with *M. bovis* are not available. Infection of the uterus and genital tract of cattle with *M. bovis* has occurred, albeit rarely, including cases of generalised infection where almost every organ in the body was infected (Cousins et al. 2004). Thus there is a risk of contamination of oocytes or embryos during collection.

Consequently, the OIE Code Article 4.7.14 ranks *M. bovis* as an IETS Category 4 disease, that is, a disease for which studies have been done, or are in progress, that indicate: that no conclusions are yet possible with regard to the level of transmission risk; or the risk of transmission via embryo transfer might not be negligible even if the embryos are properly handled according to the IETS Manual between collection and transfer (OIE 2016f).

4.15.4 Current biosecurity measures

Australia has animal biosecurity measures for *M. bovis* for bovine semen and in vivo derived embryos from the United States and in vivo derived embryos from Canada. Although disease specific testing for tuberculosis is not listed in the Canadian bovine semen import conditions, it is a requirement under Canadian legislation that bulls test negative for tuberculosis before entering the semen collection centre.

The Australian animal biosecurity measures require certification of herd freedom from bovine tuberculosis. Australia recognises the competent authorities' certification of area/herd freedom from bovine tuberculosis in Canada and the United States.

4.15.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by *Mycobacterium bovis*:

- Bovine TB is an OIE-listed disease and is nationally notifiable in Australia.
- Bovine TB is not present in Australia but is present in isolated areas in Canada and the United States.
- Bovine TB is an infectious disease affecting mainly cattle but it can be transmitted to all warm-blooded vertebrates, including humans.
- *M. bovis* can infect semen of donor bulls intrinsically and extrinsically.
- *M. bovis* can cause generalised infection. Consequently, there is a risk of oocytes and embryos becoming infected.

- Diagnostic testing of donors or donor herds not recognised free from bovine TB does not provide adequate risk management against risk of entry, establishment and spread of bovine tuberculosis via germplasm in Australia.

4.15.6 Conclusion

Based on the preceding factors, it was concluded that the overall risk of bovine TB associated with importing bovine germplasm has not changed and animal biosecurity measures are required for bovine in vitro produced embryos.

The following biosecurity measures provide appropriate risk management.

- For bovine in vitro produced embryos from Canada and the United States:
 - During the 30 days prior to, and at the time of, each collection of oocytes, the donor cows were from a herd that was located in a country or zone free from bovine tuberculosis in accordance with the OIE Code and the herd was certified free by the Veterinary Authority. (Note: for the United States, this includes donor cows from a herd that was located in a State or zone officially free from bovine tuberculosis and the herd was recognized as officially free of tuberculosis by USDA-APHIS in accordance with the USDA Tuberculosis Eradication Program.)

4.16 Contagious bovine pleuropneumonia

4.16.1 Background

Contagious bovine pleuropneumonia (CBPP) is an infectious bacterial disease of cattle and occasionally of water buffalo (*Bubalus bubalis*) caused by the bovine biotype of *Mycoplasma mycoides* subsp. *mycoides* small-colony type (SC) (*M. mycoides* SC). The disease can be acute, subacute or chronic and is characterised by a serofibrinous pleuropneumonia and severe pleural effusion (eds Coetzer & Tustin 2004). Under natural conditions, CBPP occurs in cattle of the species *Bos* and allied animals including buffalo, yak, bison and even reindeer (European Commission 2001) and *M. mycoides* SC is not transmissible to other species (Brandao 1995).

CBPP is widespread in Africa with endemic infections extending throughout the pastoral herds of much of western, central, and eastern Africa, with Angola and northern Namibia in southern Africa. CBPP has not been reported in Australia since 1967, Canada since 1876 and the United States since 1892 (OIE 2016b).

CBPP is an OIE-listed disease (OIE 2016d). In Australia, it is a notifiable disease and is classed as an EADRA Category 3 disease. An EADRA category 3 disease is a disease that has the potential to cause significant national socio-economic consequences through its impact on international trade, market disruptions involving two or more states and severe production losses to affected industries. Category 3 diseases have minimal or no effect on human health or the environment (AHA 2010; Department of Agriculture and Water Resources 2016).

4.16.2 Technical information

Pathogenesis

Following inhalation of infective droplets the organism invades the lungs causing acute lobar pneumonia and pleurisy. Bacteraemia follows, leading to localisation of the organism in numerous sites including the kidneys and brain. This results in high morbidity and mortality. Of major importance is thrombosis of the pulmonary vessels, probably before the development of pneumonic lesions. The mechanism of the development of this thrombosis is not understood (Radostits et al. 2007).

High mortality rates occur where disease is not managed and results from anoxia and septicaemia. Under natural conditions a proportion of animals in a group do not become infected, either because of natural immunity or because they are not exposed to a sufficiently large infective dose. These animals could show a transient positive reaction to the CFT. Approximately 50% of the infected animals go through a mild form of the disease and show clinical signs (Radostits et al. 2007).

Clinical signs

The incubation period is generally 3–6 weeks, but could be as long as four months. There is considerable variation in the severity of clinical disease from hyperacute to acute, subacute to chronic forms.

In calves up to six months old, CBPP manifests as polyarthritis, with lameness and swelling of affected joints.

In older animals the acute form manifests with sudden onset of marked pyrexia (40 °C), anorexia, severe depression and rapid respiration. This is followed by dry coughing which

becomes severe. The animals are reluctant to move and stand with elbows out, back arched and head extended. Respirations are shallow, rapid accompanied by expiratory grunt. The mortality rate from acute CBPP might be up to 50% and death usually occurs within three weeks of the onset of clinical signs. Many become chronic carriers (Geering, Forman & Nunn 1995b).

Some recovered animals may appear clinically normal but an inactive sequestrum sometimes forms in the lung, with a necrotic centre of sufficient size to produce a toxæmia causing ill thrift, a chronic cough, and mild respiratory distress on exercise. These sequestra break down when the animal is exposed to environmental stress and cause an acute attack of the disease (Radostits et al. 2007).

Epidemiology

The three factors which are of significance in the rate of spread of the disease are: closeness of contact, intensity of infection, and the number of susceptible animals.

Natural transmission of CBPP occurs by droplet infection from either cattle with clinical disease or from subclinical carriers actively excreting the organism to susceptible animals in close contact (Hudson 1971).

M. mycoides SC is present in large numbers in bronchial secretions, nasal discharges and exhaled air of infected animals. Dissemination of infection occurs most easily in closely stabled or trucked animals. Aerosols containing infected droplets can spread the disease over distances of 20 metres or more. Direct contact between susceptible and diseased animals is believed to be mandatory for transmission. The infection is not transmitted to healthy animals via fomites, ingestion of infected fodder or direct exposure to diseased organs of animals suffering from CBPP (Thiaucourt, van der Lugt & Provost 2004).

M. mycoides SC is also present in the urine of severely affected cattle. A 'urinary tract to nose' route of transmission or the spread of infection through aerosols of urine droplets thus appears to be possible (Masiga, Windsor & Read 1972; Scudamore 1976).

Several attenuated vaccines have been developed for protection against CBPP but none have provided complete protection.

Diagnosis

Laboratory confirmation of a presumptive diagnosis of CBPP can be achieved by tests designed to detect live *M. mycoides* SC, *M. mycoides* SC antigen, *M. mycoides* SC genetic materials or antibodies against *M. mycoides* SC.

Serological tests including a competitive-ELISA and CFT that detect *M. mycoides* SC antibodies, are the OIE prescribed tests for international trade (OIE 2016c). The CFT has significant limitations regarding sensitivity, whereas the cELISA is considered to be highly specific and sensitive, especially for herd tests. Tests on individuals may not identify those that are not sero-positive in the early stage of disease or in the chronic stage of disease.

4.16.3 Transmission in germplasm

Semen

Published research or reports on venereal transmission of *M. mycoides* SC are not available. However, *M. mycoides* SC has been isolated from semen of bulls with seminal vesiculitis

(Stradaioli et al. 1999) and semen and sheath washings from a clinically healthy bull (Goncalves 1994). Thus there is a probability of venereal transmission in bovine semen.

Embryos

Published research or reports of *M. mycoides* SC in embryos are not available. Given that *Mycoplasma* spp. have been isolated from bovine genital tracts (Pfutzner and Sachse, 1996), there is a probability of infection in bovine in vivo derived and in vitro produced embryos, even though, according to the OIE Code Article 4.7.14, the IETS has not yet ranked *M. mycoides* SC.

4.16.4 Current biosecurity measures

Australia has animal biosecurity measures for *M. mycoides* SC for bovine semen and in vivo derived embryos from Canada and the United States.

The animal biosecurity measures require certification of country freedom from CBPP as defined by the OIE Code for both bovine semen and in vivo derived embryos (OIE 2016f).

4.16.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by *M. mycoides* SC:

- CBPP is an OIE-listed disease and is nationally notifiable in Australia.
- *M. mycoides* SC is not present in Australia, Canada and the United States.
- CBPP is an infectious and contagious respiratory disease of Bovidae (cattle and buffalo).
- It is manifested by anorexia, fever and respiratory signs such as dyspnoea, polypnoea, cough and nasal discharges in bovines.
- Vaccination and serological tests of donors or donor herds do not provide adequate risk management against risk of entry, establishment and spread of CBPP in Australia.
- There is a risk of transmission via bovine semen and in vivo derived and in vitro produced embryos.

4.16.6 Conclusion

Based on the preceding factors, it was concluded that the overall risk of *M. mycoides* SC infection associated with importing bovine germplasm has not changed, and is similar for bovine in vitro produced embryos. Hence animal biosecurity measures that applied to bovine semen and bovine in vivo derived embryos will also apply for bovine in vitro produced embryos from Canada and the United States.

The following biosecurity measures provide appropriate risk management.

For bovine in vitro produced embryos from Canada and the United States:

- At the time of, and for 30 days after, each oocyte collection for this consignment, Canada/the United States was officially recognised by the OIE as a contagious bovine pleuropneumonia free country.

4.17 Leptospirosis

4.17.1 Background

Leptospirosis is a contagious disease of animals and humans caused by the spirochaete *Leptospira* spp. The bacteria can cause polymorphic disease conditions in domestic animals, wildlife and humans. Infections range from asymptomatic or subclinical to acute and fatal.

Leptospirosis has a world-wide distribution with many serovars recognised globally. Only a limited number of serovars are endemic to a region.

Leptospira spp. are currently classified in two ways:

- 1) on the basis of agglutinating antigens into over 250 serovars (svs) contained within 23 serogroups
- 2) on the basis of DNA studies with all 250 plus serovars placed into eight genomospecies.

For this review, the first classification system is used, and for brevity, each species will be named according to the serovar (sv).

In Australia, clinical leptospirosis in cattle is usually due to *L. borgpeterseni* sv *hardjo*, *L. interrogans* svs *pomona* and *zanoni* (McClintock et al. 1993). Sv *zanoni* is zoonotic in the tropics, the maintenance hosts being rats and small marsupials, and svs *hardjo* and *pomona* zoonotic in the temperate regions of Australia.

Globally, svs *hardjo* and *pomona* are recognised as the main cause of clinical leptospirosis in cattle, cattle being regarded as the maintenance host for sv *hardjo* and pigs and occasionally cattle and sheep the maintenance hosts for sv *pomona* (Elder et al. 1986).

Leptospirosis is neither an OIE-listed disease (OIE 2016d) nor nationally notifiable in Australia (Department of Agriculture and Water Resources 2016).

4.17.2 Technical information

Pathogenesis

Infection usually occurs directly through mucous membranes or through abraded or water-softened skin. The leptospires appear in the blood 4–10 hours after infection and may remain detectable in blood from only a few hours to seven days. During this period, leptospires enter and replicate in various tissues, including the liver, spleen, kidneys, reproductive tract, eyes, and central nervous system.

Leptospires are not pyogenic bacteria as such, as they do not directly cause inflammatory reactions but do so indirectly through secondary tissue reaction. Consequently, leptospires can adhere to renal tissues without causing cell damage thus enabling them to survive in the kidneys for long periods. Carriers develop, with leptospires being shed in the urine for weeks to many months after infection. In maintenance hosts, leptospires are sometimes found in the genital tract and, less commonly, in the cerebrospinal fluid and vitreous humour of the eye.

Clinical signs

Leptospirosis in cattle is usually subclinical. Serological titres vary considerably in peak and duration. Leptospires may be excreted in urine, often intermittently, for up to 18 months after infection. Sv *hardjo* can cause a sudden decrease in milk production lasting two weeks and

flaccid or atypical mastitis in cows (Ellis 2007). *Sv pomona* can cause haemolytic disease and haemoglobinuria in calves, with interstitial nephritis as a sequel, and late abortion in cows and heifers (Anderson 2007; Ellis 2007).

Epidemiology

Transmission can occur as a result of direct or indirect contact with infected animals carrying leptospires. Direct transmission is rare in accidental hosts, especially humans. Congenital transplacental infection, including non-venereal, environmentally acquired infection of pregnant females, can occur as can venereal infection (Faine et al. 1994).

Diagnosis

Serological testing is the most widely used means for diagnosing leptospirosis, with the microscopic agglutination test (MAT) being the standard serological test. However, the MAT has limitations, especially in the diagnosis of chronic infection in individual animals and in the diagnosis of endemic infections in herds. Leptospirosis may cause animals to abort or become renal/genital carriers with MAT titres below the widely accepted minimum significant titre of 1/100 (final dilution) (Ellis, O'Brien & Cassells 1981).

The ELISA is also used for detection of antibodies against leptospires, particularly in cases of recent infections, and in cattle health schemes, either as tests for *sv hardjo* on individual animal blood or milk or as bulk milk tank tests. Vaccinated animals may be positive in some ELISAs, thus complicating interpretation of the results (OIE 2016c).

4.17.3 Transmission in germplasm

Semen

Leptospires (*sv hardjo*) have been recovered from the kidney, seminal vesicle, epididymis and testis of naturally infected bulls (Philpott 1993) and from semen of naturally infected bulls (Heinemann et al. 1999; Magajevski et al. 2005; Sleight 1965) (Masri et al. 1997). Seminal transmission is rarely reported, with one report involving fresh semen or semen extended without penicillin/streptomycin antibiotics (Sleight 1965). Streptomycin is no longer permissible in food-producing animals.

Artificial insemination using raw semen from an infected and leptospiruric bull failed to transmit the disease to recipient heifers (Gale & Kingscote 1989). Intra-uterine infusion of heifers with *L. hardjo* has resulted in sero-conversion but did not affect clinical health and pregnancy rates and there was no evidence of pathogens on embryos or in the uterus (Vahdat et al. 1983).

There has been no biosecurity measures for ruminant semen from infected countries and no reports of infection arising from these importations into Australia.

Embryos

Leptospires can infect the reproductive tract of cows, including oocytes and embryos. For embryos, leptospires were observed penetrating the zona pellucida and infect the embryonic cells of domestic animals in vitro (Bielanski & Surujballi 1998). Thus the sequential IETS washing procedure with media free of antibiotics did not render infected in vivo derived or in vitro produced embryos free of the leptospires (Bielanski 1998; Bielanski & Surujballi 1998).

The presence of *sv hardjo* in the in vitro produced system had no detrimental effect on fertilisation rates or on embryonic development to the blastocyst stage. Thus it is possible to obtain transferable stage embryos from oocytes recovered from infected donors and from oocytes exposed in vitro to the *Leptospira* spp. While both in vivo derived and in vitro produced embryos may be associated with the microorganism and antibiotics used may destroy some of the leptospires, the transmission of the disease is unlikely to occur because the infectious dose required is much higher than the numbers found in the embryos (Bielanski 1998; Bielanski & Surujballi 1998).

There have been no biosecurity measures for ruminant embryos from infected countries and no reports of infection arising from these importations into Australia.

4.17.4 Current biosecurity measures

Australia has no animal biosecurity measures for *Leptospira* spp. for bovine semen and in vivo derived embryos from Canada and the United States. It should be noted that semen donors are currently tested for leptospirosis at the semen collection centres in both countries.

4.17.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by Leptospirosis:

- Leptospirosis is not an OIE-listed disease and is not nationally notifiable in Australia.
- *Leptospira* spp., in particular *svs hardjo* and *pomona* are present in Australia, Canada and the United States.
- Seminal transmission has been reported but appears to be a rare event.
- *Leptospira* spp. can be present in frozen bovine semen and embryos, most likely at levels well below the infective dose.
- The sensitivity of the MAT is poor, in many cases not detecting carriers.
- Leptospirosis has not been associated with germplasm imported into Australia from infected countries.

4.17.6 Conclusion

Based on the preceding factors, it was concluded that the risk of entry, exposure, spread, establishment and consequences are extremely low and risk management measures are not warranted for bovine in vitro produced embryos from Canada and the United States.

4.18 Bovine anaplasmosis

4.18.1 Background

Anaplasmosis, which is caused by the rickettsia *Anaplasma marginale* and, less commonly, *A. caudatum*, is an infectious but non-contagious tick-borne disease characterized by fever, progressive anaemia, icterus and sometimes sudden death in cattle. The closely related *A. centrale* is of low pathogenicity and is widely used as the antigenic component used in live anaplasma vaccines.

Bovine anaplasmosis generally occurs in the tropical and subtropical regions of the world. It is present in the cattle tick areas of Australia and the United States. Canada has experienced sporadic cases of anaplasmosis since 1968 (Howden et al. 2010).

Cattle of all ages are susceptible to infection by *A. marginale* and severity of disease usually increases with age. Other ruminants such as buffalo, bison, deer and antelopes may also become infected (Aubry & Geale 2011).

Bovine anaplasmosis is an OIE-listed disease (OIE 2016d) and is nationally notifiable in the cattle tick-free areas of Australia (Department of Agriculture and Water Resources 2016).

4.18.2 Technical information

Pathogenesis

After infection followed by an incubation period ranging from 7 to 60 days with an average of 28 days, *A. marginale* invades the erythrocytes where it replicates cyclically (Kocan et al. 2010). During acute infection, up to 10 to 90% of erythrocytes may be parasitised. The infected erythrocytes are removed by the reticuloendothelial system (Aubry & Geale 2011).

Clinical signs

Clinical signs of acute infection is characterised by anaemia and icterus. There is no haemoglobinaemia and haemoglobinuria as the infected erythrocytes are phagocytised by the bovine reticuloendothelial system. Other signs include fever, weight loss, abortion, lethargy and death (Kocan et al. 2010). The severity of disease is age dependent, with clinical disease becoming more apparent with age.

Epidemiology

Transmission of *A. marginale* is via tick bites, or the mechanical transfer of fresh erythrocytes from biting flies or surgical equipment such as needles, or dehorning, castration or tattooing equipment (Aubry & Geale 2011).

Once cattle become infected, they remain persistently infected carriers for life, whether or not they develop clinical disease. Throughout the carrier's remaining life, there are cycles of 10–14 day periods of fluctuating numbers of circulating erythrocytes infected with the parasite (Kocan et al. 2010).

Diagnosis

A. marginale can be identified by direct microscopic examination of blood smears stained with geimsa stain in clinically affected animals. There are also serological tests for detecting carriers, such as the cELISA and card agglutination. PCR tests have been used experimentally (OIE 2016c).

4.18.3 Transmission in germplasm

Semen

Infection of bulls with *A. marginale* causes marked deterioration in semen quality but the parasite is not known to infect semen (Swift, Reeves & Thomas 1979). It is generally recognised that the risk of transmission via semen is unlikely (Eaglesome & Garcia 1997).

Embryo

Published research or reports of *A. marginale* transmission via bovine in vivo derived or in vitro produced embryos are not available. However, there was a report of clinical anaplasmosis in heifers that received in vivo derived embryos from a cow at an embryo transfer station in the United States. The source of infection could not be identified as the donor cow originated from the same herd as the heifers, and these cattle were kept in a region free from bovine anaplasmosis (Coy & Schillhorn van Veen 1984).

The OIE Code Article 4.7.14 currently ranks bovine anaplasmosis as an IETS Category 4 disease for in vivo derived embryos, that is, a disease for which studies have been done, or are in progress, that indicate: that no conclusions are yet possible with regard to the level of transmission risk; or the risk of transmission via embryo transfer might not be negligible even if the embryos are properly handled according to the IETS Manual between collection and transfer (OIE 2016f).

4.18.4 Current biosecurity measures

Australia has no animal biosecurity measures for bovine anaplasmosis for bovine semen, in vivo derived and in vitro produced embryos from Canada and the United States.

4.18.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by bovine anaplasmosis:

- Bovine anaplasmosis is an OIE-listed disease and is nationally notifiable in the tick free areas of Australia.
- *A. marginale* has been reported in the tick zone of Australia, the United States and occasionally in Canada.
- *A. marginale* is a blood parasite that causes anaemia and icterus.
- There is no evidence that *A. marginale* can be present in bovine semen and embryos despite bovine anaplasmosis being listed as an IETS Category 4 disease.
- The OIE Code provide no guideline for certification for both bovine semen and embryos.

4.18.6 Conclusion

Based on the preceding factors, it was concluded that animal biosecurity measures for *A. marginale* are not warranted for bovine in vitro produced embryos from Canada and the United States.

4.19 Trichomoniasis

4.19.1 Background

Trichomoniasis is a venereal disease of cattle caused by the protozoan parasite *Tritrichomonas foetus*. In cows and heifers, it can cause infertility, early abortion and pyometra but in the infected bull, a symptomless carrier state occurs with *T. foetus* being found on the penis and preputial membranes.

Trichomoniasis occurs world-wide, particularly among range cattle. High herd prevalence has been reported in areas of North America where natural breeding is practised (Eaglesome & Garcia 1997). Bovine trichomoniasis is an uncommon disease in NSW and the other states of southern Australia. However, the disease is endemic to northern Australia where a significant proportion of bulls may be infected.

Trichomoniasis is an OIE-listed disease (OIE 2016d) but not nationally notifiable in Australia (Department of Agriculture and Water Resources 2016).

4.19.2 Technical information

Pathogenesis

Transmission is primarily by coitus, but mechanical transmission by insemination instruments or by gynaecological examination can occur (Yule, Skirrow & BonDuran 1989). Bulls are the main reservoir of the disease as they tend to be long-term carriers, whereas most cows clear the infection spontaneously.

The protozoa attach to the surfaces of epithelial cells lining the reproductive tract. In heifers and cows, these include the vagina, uterus, and oviduct. *T. foetus* can be found in secretions from these sites, including the mild mucopurulent discharge associated with vaginitis and endometritis. In bulls, the protozoa infect the penis and preputial membranes, localising in the secretions (smegma) of the epithelial lining of the penis, prepuce, and distal portion of the urethra (Rhyan et al. 1999).

Clinical signs

In bulls, the site of infection is primarily the preputial cavity (Rhyan et al. 1999; Yule, Skirrow & BonDuran 1989). There is little or no clinical manifestation. In older bulls, spontaneous recovery rarely occurs, and they become permanent source of infection in herds. In younger bulls under 3–4 years old, infection may be transient. The parasite does not affect either semen quality or sexual behaviour.

In cows, the infection invades the vagina, uterus and oviducts, causing vaginitis, embryonic death, abortions and infertility which may last for several months. Sequelae include placentitis leading to early abortion (1–16 weeks), uterine discharge, and pyometra. On a herd basis, cows may, following infection, exhibit irregular oestrous cycles, uterine discharge, pyometra, or early abortion. Cows usually clear their infection and generally become immune, at least for that breeding season (Yule, Skirrow & BonDuran 1989).

Epidemiology

Although the parasite can survive in diluted semen and through the freezing process, the probability of transmitting infection through AI is not known. However, testing and culling of infected bulls, especially when used for artificial breeding, has reduced the incidence of infection

in many areas. Where bulls are used for natural mating under extensive rangelands conditions the prevalence is high.

Cattle rarely develop circulating antibodies to the parasite, as *T. foetus* does not penetrate adult tissues (although it may invade the placenta and the foetus). Consequently, there is no effective blood test (Yule, Skirrow & BonDuran 1989).

Diagnosis

A presumptive diagnosis can be made by direct examination for the living motile organisms in preputial washings or scrapings or after culture (washings/scrapings) for up to 7–10 days. A PCR has been developed to provide high test sensitivity and specificity and is useful for differentiating from other trichomonads (Parker et al. 2003).

Confirmation of the diagnosis requires isolation of *T. foetus*, which may be difficult to differentiate from other trichomonads resident in the digestive tract. Diagnostic efforts are directed at bulls, because they are the most likely carriers. Because *T. foetus* is present in small numbers in the preputial cavity, with some concentration in the fornix and around the glans penis, serial testing is recommended to improve the sensitivity of tests (OIE 2016c).

4.19.3 Transmission in germplasm

Semen

T. foetus is a sexually transmissible disease, although transmission via artificial insemination is rare. The parasite can survive in fresh or frozen, whole or diluted semen (Yule, Skirrow & BonDuran 1989). With regards to the use of semen in in vitro produced embryo production, *T. foetus* has an inhibitory effect on sperm motility during swim-up and in vitro fertilisation (Bielanski, Ghazi & Phipps-Toodd 2004).

Embryo

Under experimental conditions, *T. foetus* adheres rapidly to the cumulus cells and zona pellucida of oocytes, causing severe damage to the zona pellucida, cumulus cells and the oocytes (Benchimol, da Silva Fontes & Burla Dias 2007) and also damages the trophoblastic cells of hatched blastocysts (Bielanski, Ghazi & Phipps-Toodd 2004). Thus there is a risk of infection and transmission if the cows were naturally mated with infected bulls. Generally, embryos are collected from cows that have not been joined to bulls but were inseminated with semen from donors that have tested negative for *T. foetus*.

Consequently, the OIE Code Article 4.7.14 ranks trichomoniasis as an IETS Category 4 disease for in vivo derived embryos, that is, a disease for which studies have been done, or are in progress, that indicate: that no conclusions are yet possible with regard to the level of transmission risk; or the risk of transmission via embryo transfer might not be negligible even if the embryos are properly handled according to the IETS Manual between collection and transfer (OIE 2016f).

However, with regards in vitro produced embryos, the parasites could not survive the in vitro culture conditions. Up to 10^6 /ml *T. foetus* was added at start of in vitro-fertilisation or 24 hours into in vitro culture with no motile protozoa remaining after 18 hours of in vitro fertilisation or 72 hours of in vitro culture. Thus the risk of transmission of trichomoniasis is unlikely (Bielanski, Ghazi & Phipps-Toodd 2004).

4.19.4 Current biosecurity measures

Australia has animal biosecurity measures for *T. foetus* for bovine semen, but not for in vivo derived embryos from Canada and the United States.

4.19.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by trichomoniasis:

- Trichomoniasis is an OIE-listed disease but is not nationally notifiable in Australia.
- *T. foetus* is present in Australia, Canada and the United States.
- *T. foetus* can be present in bovine semen and in vivo derived embryos.
- The source of risk for in vivo derived embryos is semen from infected bulls.
- *T. foetus* does not survive the in vitro culture conditions for in vitro produced embryos.

4.19.6 Conclusion

Semen used for fertilising in vitro produced embryos must be sourced from semen collection centres that meet the standards specified in the OIE Code, that is, the semen donors were tested free from *T. foetus*. Based on this and the preceding factors, it was concluded that no further animal biosecurity measures are warranted for *T. foetus* for bovine in vitro produced embryos from Canada and the United States.

4.20 Bovine spongiform encephalopathy

4.20.1 Background

Bovine spongiform encephalopathy (BSE), one of a group of diseases known as transmissible spongiform encephalopathies, is a progressive fatal disease of the nervous system of cattle and rarely goats. BSE has been linked to neurological diseases in cats and humans following consumption of beef products contaminated with infected tissues and, in the latter case, by contaminated medical devices. The cause of BSE is associated with the presence of a disease-specific partially protease-resistant, misfolded isoform of a membrane protein, often abbreviated as PrP^{Sc}, in the nervous tissue. There is increasing evidence that there are different strains of BSE: the typical BSE strain responsible for the outbreak in the United Kingdom and two atypical strains (H and L strains).

In the United Kingdom where BSE was first recognised and defined in 1986, the typical BSE strain accounted for more than 180,000 cases diagnosed in cattle between 1987 and 2007, representing 97% of all cases reported world-wide. Both Canada and the United States reported cases, all of which were detected by their ongoing surveillance programs. All three of the United States-born BSE cases and two of the 20 Canadian-born BSE cases were 10 years of age or older and caused by atypical BSE strains. Of these five older North American cases, three were linked to an atypical BSE strain known as the H-type. The strain type for the other two older North American cases, a 13-year-old BSE-infected Canadian cow and a 10-year-old BSE-infected US cow, were identified as the L-type (CDC 2016).

BSE has been reported in a total of 23 other countries in Europe and Asia. The implementation of appropriate control measures by many countries has resulted in a decline of BSE worldwide diagnosed in cattle from 37,000 cases in 1992 to less than 20 cases per year in the past three years (OIE 2016a). The OIE currently recognises the United States as a country with negligible risk of BSE and Canada as a country with a controlled risk of BSE.

BSE is an OIE-listed disease for which the OIE has established official recognition of the sanitary status countries and zones (OIE 2016d). In Australia, it is nationally notifiable and is classed as an EADRA Category 2 disease, that is, a disease that has the potential to cause major national socio-economic consequences through very serious international trade losses, national market disruptions and very severe production losses in the livestock industries that are involved (AHA 2010; Department of Agriculture and Water Resources 2016).

4.20.2 Technical information

Pathogenesis

In cattle, the pathogenesis of BSE determines the tissue distribution of the infective agent and the incubation period of the disease (Arnold et al. 2009; van Keulen, Bossers & Van Zijderveld 2008; Wells, Ryder & Hadlow 2007).

BSE occurs when prions are ingested in contaminated feedstuffs and cross the epithelium of the distal ileum. Infection of tonsils is rare. BSE prions then replicate in Peyer's patches and other lymphoid tissue associated with the gut. Experimental evidence has shown that infectivity develops in the distal ileum between four and six months post infection (Arnold et al. 2009).

Following replication in the distal ileum, BSE prions enter the nervous system via neural networks in the gut wall. From there, they migrate primarily via nerve tracts of the autonomic nervous system (vagus nerve) to the brain and the spinal cord and its associated dorsal root ganglia. In addition, some prions might migrate via the sympathetic gastrointestinal tract innervations (Hoffmann et al. 2007).

In field cases, BSE prions have not been detected in the bovine lymphoid system other than the lymphoid tissue directly associated with the distal ileum (Iwata et al. 2006; Terry et al. 2003). BSE prions were also absent from blood leukocytes, especially lymphocytes, and it is concluded that their transport into organs and exudates by means of these migratory cells does not occur (van Keulen, Bossers & Van Zijderveld 2008). This observation explained the absence of infectivity in exudates, skeletal muscles, spleen and other lymphatic tissues and reproductive tissues (Balkema-Buschmann et al. 2011; Buschmann & Groschup 2005).

Clinical signs

The average time from consumption of, and hence infection with, prions to onset of clinical signs typically ranges from four to five years. Symptoms include nervous or aggressive behaviour, depression, hypersensitivity to sound and touch, twitching, tremors, abnormal posture, in-coordination in movements, weight loss and/or decreased milk production. Symptoms usually last two to six months before death. Early clinical signs are often subtle and mostly behavioural, and affected animals were sometimes disposed of before suspicion of BSE could be confirmed (Ducrot et al. 2008; Wilesmith 1998).

Epidemiology

It has been established beyond reasonable doubt that transmission is mostly by feeding rendered material, especially meat-and-bone meal, from infected cattle to other cattle. Prions are highly resistant to the commercial inactivation procedures, particularly heat, during the production of rendered material. Stock feeding practices present in the UK, prior to the BSE outbreak, which involved the feeding of concentrate rations containing meat-and-bone meal to dairy calves resulted in a much higher prevalence of BSE in dairy herds compared to beef herds (Wilesmith 1998).

There is no evidence to suggest that cattle are infected with BSE from a prion contaminated environment, as is observed with the prion caused diseases of sheep and goat scrapie and chronic wasting disease in deer.

The OIE has published a list of specified risk materials (SRMs), as has the EC Scientific Steering Committee but the committee's list includes the rationale for their selection based on the substantial experimental evidence that has accumulated regarding the distribution of infectivity throughout cattle tissues. BSE infectivity has been demonstrated in the following tissues to varying degrees: brain, eyes (retina), trigeminal ganglia, the spinal cord, the dorsal root ganglia and the distal ileum and these materials are classed as SRMs. Regional lymph nodes, including those of the head have no detectable infectivity (European Commission 2002). No infectivity was detected by bioassay in mice injected both intracerebrally and intraperitoneally with material derived from reproductive tissues from confirmed cases of BSE in cattle. The reproductive tissues assayed included testis, prostate, epididymis, seminal vesicle, semen, ovary, uterine caruncle, placental cotyledon, placental fluids (both amniotic fluid and allantoic fluid), udder and milk, from confirmed cases of BSE cattle (European Commission 2002).

The OIE recommends a range of strategic measures for control, eradication and monitoring of BSE. These include implementation of prevention, risk mitigation, surveillance and rapid response measures. Preventative actions taken include the removal of SRMs from carcasses during slaughter and processing, and the prohibition of the inclusion of SRMs in animal feeds. These actions remove potentially contaminated material from the food chain (OIE 2016f).

Diagnosis

BSE may be suspected on clinical symptoms, especially in adult cattle, but diagnosis must be confirmed by the application of immunohistochemical and/or immunochemical methods to brain tissue for the detection of PrP^{Sc} in specific neuroanatomical loci in the central nervous system of affected cattle, by immunohistochemical methods in formalin-fixed material, or by immunoblotting and other enzyme immunoassay methods using unfixed brain extracts (OIE 2016c). There are currently no screening tests for live animals.

4.20.3 Transmission in germplasm

Semen

Bioassays in mice have failed to detect infectivity in testis, seminal vesicles and semen of scrapie-affected rams, or in testes, epididymis, prostate, seminal vesicles and semen of BSE affected bulls (European Commission 2002). Semen from bulls clinically affected with BSE and inseminated into clinically affected cows does not lead to transmission of BSE via the resulting embryos to the recipients or to the embryo transfer offspring (Wrathall et al. 2002). An epidemiological study involving infected and healthy bulls used for artificial insemination showed no difference in the incidence of BSE between their progenies (Wilesmith 1994).

Embryos

Studies using over 1,500 in vivo derived embryos collected from cows with clinical BSE and inseminated with semen from clinically infected bulls concluded that embryos are unlikely to carry BSE infectivity even if they have been collected at the end-stage of the disease, when the potential risk of maternal transmission is believed to be highest (Wrathall et al. 2002).

Consequently the OIE Code Article 4.7.14 ranks BSE as an IETS Category 1 disease, that is, a disease for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the in vivo derived bovine embryos are properly handled and washed between collection and transfer (OIE 2016f).

While there have been no published or reported studies examining the risk of BSE transmission via in vitro produced embryos, the evidence from semen and in vivo derived embryos and the fact that BSE infectivity could not be shown in reproductive organs and regional lymph nodes strongly indicate that in vitro produced embryos are also unlikely to carry BSE infectivity (European Commission 2002). Since 1997, world-wide over three million bovine in vitro produced embryos have been transferred without any report of BSE. Therefore the risk of BSE transmission via in vitro produced embryos is assessed to be negligible.

4.20.4 Current biosecurity measures

Australia has no animal biosecurity measures for BSE for semen and in vivo derived embryos.

4.20.5 Risk review

The following key points were drawn from the preceding information to inform the review of biosecurity risk presented by BSE prions:

- BSE is an OIE-listed disease and is a nationally notifiable in Australia.
- The OIE currently recognises Canada as a country with a controlled risk of BSE and the United States as a country with negligible risk of BSE.
- BSE is a progressive fatal disease of the nervous system of cattle and rarely, goats.
- BSE prions are absent from blood leukocytes, especially lymphocytes, and their transport into reproductive tissues by means of these migratory cells is unlikely.
- Transmission is by feeding material containing infected tissues, especially to young cattle. There is no evidence that cattle are infected with BSE from a contaminated environment or by other means.
- BSE specified risk materials (SRM) do not include reproductive organs of bulls or cows.
- Studies have shown that the risk of transmission via bovine germplasm is negligible.

4.20.6 Conclusion

Based on the preceding factors, it was concluded that the overall risk of BSE infection associated with importing bovine germplasm has not changed, is negligible, and is similar for bovine in vitro produced embryos. Hence animal biosecurity measures are not warranted for BSE for bovine in vitro produced embryos from Canada and the United States.

5 Biosecurity measures for importation of frozen bovine in vitro produced embryos from Canada

This chapter refers only to the veterinary certification requirements and does not include the general information that needs to be provided with the veterinary certificate. General information, which is in line with the OIE recommendations for health certificates and complies with Australian government legislation and the department's policies, can be found at: [Biosecurity Import Conditions \(BICON\) system](https://agriculture.gov.au/import/online-services/bicon) (agriculture.gov.au/import/online-services/bicon) and includes details about the import permit, the importer, male and female donors, semen, oocyte collection facilities and the in vitro produced embryo processing laboratory. The general information relevant to importing frozen bovine in vitro produced embryos will be similar to those for importing bovine semen and in vivo derived embryos.

5.1 Embryo production team(s) and team veterinarian(s)

Note: *The embryo production team is a group of competent veterinarians and technicians and includes the Team Veterinarian, to perform the collection and processing of ovaries/oocytes and the production and storage of in vitro produced embryos.*

5.1.1 The embryo production team veterinarian or the embryo production team was approved by the CFIA for export of bovine in vitro produced embryos.

5.1.2 The Team Veterinarian is:

- i) certified by the Canadian Embryo Transfer Association as a competent embryo transfer practitioner
- ii) deemed to be competent in the production of in vitro produced embryos.

5.1.3 The embryo production team was supervised by the Team Veterinarian.

5.1.4 The Team Veterinarian was responsible for all team operations which include the hygienic collection of oocytes and all other procedures involved in the production of embryos intended for international movement.

5.1.5 The embryo production team personnel were adequately trained in the techniques and principles of disease control. High standards of hygiene were practised to preclude the introduction of infection.

5.1.6 The embryo production team had adequate facilities and equipment for:

- i) collecting ovaries and/or oocytes
- ii) processing of oocytes and production of embryos at a permanent or mobile laboratory
- iii) storing oocytes and/or embryos.

These facilities need not necessarily be at the same location.

- 5.1.7 The embryo production team have kept a record of its activities, which should be maintained for inspection by the CFIA for a period of at least two years after the embryos have been exported.

5.2 Oocyte collection facility

Note: *The oocyte collection facility is the premises consisting of an oocyte recovery area and a permanent or mobile laboratory for the processing of oocytes and in vitro maturation before transporting to the in vitro produced embryo processing laboratory. The premises may also include the in vitro produced embryo processing laboratory.*

The oocyte recovery area is the area dedicated to the ultrasonographically guided aspiration of oocytes and includes facilities for the safe handling of donor cows.

- 5.2.1 The oocyte collection facility:

- i) was on a property not subject to any restriction or quarantine measure with respect to contagious and infectious animal diseases
- ii) was under the supervision of the Team Veterinarian
- iii) was built and maintained in accordance with the recommendations in the current IETS Manual to permit the sanitary collection, handling and processing of the oocytes for maturing
- iv) was subjected to, and passed, inspection at least once a year by an approved veterinarian in the Embryo Production Team
- v) was subjected to review by the CFIA confirming approval at least once a year.

- 5.2.2 Only animals associated with oocyte collection and meeting health requirements as specified in this document were permitted to enter the oocyte recovery area during collection of oocytes for processing to in vitro produced embryos for export to Australia.

5.3 Oocyte donors

- 5.3.1 Only live animals permanently identified according to an identification system recognised by the CFIA were used for oocyte collection.
- 5.3.2 To the knowledge of the Team Veterinarian, donors showed no clinical signs of contagious and infectious diseases for 30 days prior to, at the time of, and for 30 days after, each collection.
- 5.3.3 The Team Veterinarian or another veterinarian authorised by the Team Veterinarian inspected each female donor on each day that the oocytes were collected for this consignment and certified the donor to be free of clinical signs from contagious and infectious diseases.
- 5.3.4 Donors resided in Canada for at least 90 days prior to oocytes collection for this consignment.

5.4 Oocyte collection, processing and in vitro maturation

Note: *An oocyte collection is defined as oocytes collected during a single ovum pickup from a live donor.*

- 5.4.1 Only oocytes from the same female donor were washed and processed together.

- 5.4.2 All equipment/materials were disposed of and replaced with new items, or sterilised or disinfected in accordance with the current IETS Manual, before use and between different donors.
- 5.4.3 No oocytes of a lesser health status were processed within the laboratory at the same time as the germplasm for this consignment.
- 5.4.4 Any biological product of animal origin, including media constituents, used in oocyte recovery, maturation, washing and storage presented no animal disease risk. Media were sterilised prior to use by approved methods in accordance with the current IETS Manual and handled in such a manner as to ensure that sterility is maintained. Antibiotics were added to all fluids and media as recommended in the current IETS Manual.

5.5 Transport of oocytes from oocyte collection centre

- 5.5.1 The oocytes were processed, stored and transported to the in vitro produced embryo processing laboratory in a hygienic manner in accordance with recommendations of the current IETS Manual.
- 5.5.2 Only oocytes from the same individual donor were stored together in the same ampoule, vial or straw.
- 5.5.3 Ampoules, vials or straws were capped or sealed before transport.
- 5.5.4 Where a third party was used for transport, the storage container was sealed at the oocyte collection centre by the Team Veterinarian or an approved veterinarian who is a member of the embryo production team and the seal was not broken until receipt by the Team Veterinarian or a member of the embryo production team at the in vitro produced embryo processing laboratory.

5.6 Semen donors

- 5.6.1 The semen donor must be resident in Canada for 90 days prior to the collection of semen used to fertilise the oocytes in this consignment.

5.7 Semen

- 5.7.1 Only semen certifiable for export to Australia was used to fertilise the oocytes. Evidence was provided by the Team Veterinarian to CFIA for review.
- 5.7.2 If the semen was collected in another country, the semen importer provided a copy of certification from the country of origin to CFIA as evidence that the semen met Australian import requirements.

5.8 In vitro produced embryo processing laboratory

Note: *The in vitro produced embryo processing laboratory is the facility at which the in vitro produced embryos were processed through, at minimum, in vitro fertilisation, in vitro culture, embryo washing and freezing.*

- 5.8.1 This facility:
 - i) was on a property not subject to any restriction or quarantine measure with respect to contagious or infectious animal disease
 - ii) was under the supervision of the Team Veterinarian

- iii) is a permanent structure that was built and maintained in accordance with the recommendations of the current IETS Manual
- iv) was subjected to, and passed, inspection at least once a year by the Team Veterinarian
- v) was subjected to review confirming approval at least once a year by the CFIA.

5.9 Production and storage of embryos

- 5.9.1 During the production of embryos for export to Australia and prior to their storage, no oocytes or embryos of a lesser health status were processed at the same time using the same equipment and materials.
- 5.9.2 All equipment/materials were disposed of and replaced with new items, or disinfected in accordance with the recommendations of the current IETS Manual between different donors.
- 5.9.3 Any biological product of animal origin, including co-culture cells and media constituents, used in fertilisation, culture, washing and storage presented no animal disease risk. Media were sterilised prior to use by approved methods in accordance with the current IETS Manual and handled in such a manner as to ensure that sterility is maintained. Antibiotics were added to all fluids and media as recommended in the current IETS Manual.
- 5.9.4 Cleaning, and sterilisation or disinfection of, equipment were carried out in accordance with the recommendations of the current IETS Manual.

5.10 Embryos

- 5.10.1 The embryos were handled in accordance with the current IETS Manual:
 - i) All embryos are identified and can be traced to the male and female donors.
 - ii) Only embryos from the same female donor were washed together, and no more than ten embryos were washed at any one time.
 - iii) The zona pellucida of each embryo, before washing, was examined over its entire surface area at not less than 50X magnification to ensure that it is intact and free of adherent material.
 - iv) The embryos were washed at least ten times with at least 100-fold dilutions between each wash, and a new sterile micropipette was used for transferring the embryos through each wash.
 - v) The standard washing procedure includes additional washes with the enzyme trypsin.
 - vi) If performed, micromanipulation for biopsy for genetic testing was carried out only on embryos with intact zona pellucida after the standard washing procedure, and in suitable laminar-flow facilities which were properly cleaned and disinfected between batches.

5.11 Diagnostic testing

- 5.11.1 The samples were collected by veterinarians approved by the CFIA for export certification.

- 5.11.2 Tests for disease were carried out at a laboratory approved by the competent authority to perform the required test.
- 5.11.3 The tests were conducted in accordance with the current OIE Manual.
- 5.11.4 The test reports provided to CFIA to support certification must display the dates of sampling for the tests required, the type of test used and the test results. This information must be contained in a table against donor information, annexed to the health certificate, and verified and certified correct by the CFIA certifying officer.

5.12 Disease freedom

- 5.12.1 At the time of, and for 30 days after, each oocyte collection for this consignment, Canada was officially recognised by the OIE as a:
 - i) foot and mouth disease free country where vaccination is not practised
 - ii) contagious bovine pleuropneumonia free countryAND meets the current OIE Code Article definitions for country freedom from:
 - iii) lumpy skin disease
 - iv) Rift Valley fever
 - v) brucellosis due to *B. melitensis*AND was recognised by Australia as a country free from foot and mouth disease where vaccination is not practised.

5.13 Vesicular stomatitis

- 5.13.1 During the 30 days prior to, and at the time of, each collection of oocytes, there was no clinical signs or reports of vesicular stomatitis at the premises where donor cows were kept and at the oocyte collection facility.

5.14 Bovine brucellosis

- 5.14.1 During the 30 days prior to, and at the time of, each collection of oocytes, the donor cows were from a herd that was located in a country or zone free from infection with bovine brucellosis (*B. abortus*) and the herd was certified free without vaccination by the CFIA in accordance with the current OIE Code.

5.15 Bovine tuberculosis

- 5.15.1 During the 30 days prior to, and at the time of, each collection of oocytes, the donor cows were from a herd that was located in a country or zone free from bovine tuberculosis in accordance with the current OIE Code and the herd was certified free by the CFIA.

5.16 Bluetongue

EITHER

- 5.16.1 Blood samples drawn from each donor:
 - i) were subjected to a cELISA test to detect antibodies to the BTV group between 28 and 60 days after each collection of oocytes with negative resultsor

- ii) were subjected to an agent identification test on a blood sample taken on the day of collection with negative results.

OR

- 5.16.2 All donors were kept in a country that was free, or seasonally free, from BTV as recognised by Australia* at least 60 days prior to, and at the time of, collection of oocytes.

(*Australia recognises Canada as a country seasonally free from BTV without testing between 1 January and 15 May, except the Okanagan Valley of British Columbia)

5.17 Epizootic haemorrhagic disease (EHD)

EITHER

- 5.17.1 Blood samples drawn from each donor:

- i) were subjected to the cELISA test to detect antibodies to the EHDV group between 28 and 60 days after each collection of the oocytes for this consignment with negative results

or

- ii) were subjected to an agent identification test on a blood sample taken on the day of collection with negative result.

OR

- 5.17.2 All donors were kept in a country that was free, or seasonally free, from EHDV as recognised by Australia* at least 60 days prior to, and at the time of, collection of oocytes.

(*Australia recognises Canada as a country seasonally free from EHDV without testing between 1 January and 15 May, except the Okanagan Valley of British Columbia)

5.18 Infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV)

- 5.18.1 For all oocytes collected from the donors:

EITHER

- i) The donor came from an IBR/IPV free herd as defined in the current OIE Code

or

- ii) The donor was subjected, with negative results, to a serological test for IBR/IPV on blood samples collected at least four weeks after each oocyte collection.

OR

- iii) The donor was kept in a herd where all eligible animals including the donor were vaccinated against IBR/IPV with a vaccine approved by the CFIA/Health Canada at least 30 days prior to collection of oocytes. The vaccine was administered as per manufacturer's instructions for vaccination and revaccination

and

- iv) A vaginal mucus sample[#] was collected from the donor immediately prior to washing/disinfecting the vagina in preparation for oocyte collection and tested for

bovine herpesvirus-1 by the qRT-PCR for bovine herpesvirus-1 with negative results.

#Double guarded culture swabs, appropriately prepared for testing samples, were used to hygienically collect samples of vaginal mucus from the donors.

5.19 Bovine viral diarrhoea virus (BVDV)

5.19.1 At the time of each collection of oocytes, each female donor gave a negative result to one of the following tests for BVDV:

i) an antigen-capture ELISA on peripheral blood leucocytes

or

ii) a monoclonal immunoperoxidase or other virus isolation test on blood or serum.

AND

5.19.2 If vaccinated, the donors were kept in a herd where all eligible animals including the donors were vaccinated against both BVDV-1 and BVDV-2 with a vaccine approved by the CFIA / Health Canada at least 30 days prior to collection of oocytes. The vaccine was administered as per manufacturer's instructions for vaccination and revaccination.

5.20 Schmallerberg viruses

5.20.1 No cases of disease caused by Schmallerberg virus has been detected or reported in Canada.

5.21 Storage and transport

5.21.1 From the time of embryo freezing until export, the in vitro produced embryos in this consignment were stored for at least 30 days:

i) in sealed sterile containers (e.g. straws, ampoules or vials) and identified in a legible and non-erasable manner as specified in the current IETS Manual. Goblets and canes were also identified as specified in the current IETS Manual

ii) EITHER

only with other bovine germplasm collected for export to Australia, or of equivalent health status

OR

with other export certifiable germplasm provided ALL straws, ampoules or vials were sealed and intact

iii) in storage or shipping containers containing only new, unused liquid nitrogen

iv) in a secure place within an approved centre or laboratory

v) under the supervision of the Team Veterinarian.

5.22 Shipping containers (liquid nitrogen shippers/tanks)

EITHER

5.22.1 The shipping container was new.

OR

- 5.22.2 Immediately prior to loading, the shipping container was emptied and inspected and any loose straws removed. The shipping container, including all surfaces in contact with the straws, ampoules or vials was then disinfected with one of the following disinfectants: 2% available chlorine (e.g. chlorine bleach), 2% Virkon or irradiated at 50 kGy.

Date of disinfection/ irradiation

Disinfectant used/ active ingredient

[The veterinary certificate must indicate the option that applies. For used shipping containers, the date of disinfection, the disinfectant used and its active chemical must be recorded on the health certificate.]

5.23 Official government seals

- 5.23.1 Under the supervision of an Official Government Veterinarian prior to export to Australia:

- i) the containers (e.g. straws, ampoules or vials) for reproductive material in this consignment were checked as being sealed
- ii) the identity of the reproductive material was checked prior to being placed into new, unused liquid nitrogen in a shipping container for export that was new or disinfected as specified in this veterinary certificate.

- 5.23.2 Only bovine reproductive material (that is, semen and/or in vivo derived embryos and/or in vitro-produced embryos) that met Australian import conditions can be transported in the same shipping container.

- 5.23.3 An official government seal was applied by an Official Government Veterinarian to the shipping container and the number or mark on the seal recorded on the certificate.

Shipping container official government seal number

6 Biosecurity measures for importation of frozen bovine in vitro produced embryos from the United States

This chapter refers only to the veterinary certification requirements and does not include the general information that needs to be provided with the veterinary certificate. General information, which is in line with the OIE recommendations for health certificates and complies with Australian government legislation and the department's policies, can be found at: [Biosecurity Import Conditions \(BICON\) system](https://agriculture.gov.au/import/online-services/bicon) (agriculture.gov.au/import/online-services/bicon) and includes details about the import permit, the importer, male and female donors, semen, oocyte collection facilities and the in vitro produced embryo processing laboratory. The general information relevant to importing frozen bovine in vitro produced embryos will be similar to those for importing bovine semen and in vivo derived embryos.

6.1 Embryo production team(s) and team veterinarian(s)

Note: *The embryo production team is a group of competent veterinarians and technicians and includes the Team Veterinarian, to perform the collection and processing of ovaries/oocytes and the production and storage of in vitro produced embryos.*

6.1.1 The embryo production team veterinarian or the embryo production team was approved by USDA-APHIS for export of bovine in vitro produced embryos.

6.1.2 The Team Veterinarian is:

- i) certified by the American Embryo Transfer Association (AETA) as a competent embryo transfer practitioner
- ii) deemed to be competent in the production of in vitro produced embryos.

6.1.3 The embryo production team was supervised by the Team Veterinarian. The Team Veterinarian was responsible for all team operations which include the hygienic collection of oocytes and all other procedures involved in the production of embryos intended for international movement.

The embryo production team personnel were adequately trained in the techniques and principles of disease control. High standards of hygiene was practised to preclude the introduction of infection.

6.1.4 The embryo production team had adequate facilities and equipment for

- i) collecting ovaries and/or oocytes
- ii) processing of oocytes and production of embryos at a permanent or mobile laboratory
- iii) storing oocytes and/or embryos.

These facilities need not necessarily be at the same location.

- 6.1.5 The embryo production team is keeping a record of its activities, which should be maintained for inspection by USDA–APHIS for a period of at least two years after the embryos have been exported.

6.2 Oocyte collection facility

Note: *The oocyte collection facility is the premises consisting of an oocyte recovery area and a permanent or mobile laboratory for the processing of oocytes and in vitro maturation before transporting to the in vitro produced embryo processing laboratory. The premises may also include the in vitro produced embryo processing laboratory.*

The oocyte recovery area is the area dedicated to the ultrasonographically guided aspiration of oocytes and includes facilities for the safe handling of donor cows.

- 6.2.1 The oocyte collection facility:

- i) was on a property not subject to any restriction or quarantine measure with respect to contagious and infectious animal diseases
- ii) was under the supervision of the Team Veterinarian
- iii) was built and maintained in accordance with the recommendations in the current IETS Manual to permit the sanitary collection, handling and processing of the oocytes for maturing.
- iv) was subjected to, and passed, inspection at least once a year by the Team Veterinarian
- v) was subjected to review by USDA–APHIS confirming approval at least once a year.

- 6.2.2 Only animals associated with oocyte collection and meeting health requirements as specified in this document were permitted to enter the oocyte recovery area during collection of oocytes for processing to in vitro produced embryos for export to Australia.

6.3 Oocyte donors

- 6.3.1 Only live animals permanently identified according to an identification system recognised by USDA–APHIS were used for oocyte collection.

- 6.3.2 To the knowledge of the Team Veterinarian, donors showed no clinical signs of contagious and infectious diseases for 30 days prior to, at the time of, and for 30 days after, each collection.

- 6.3.3 The Team Veterinarian or another veterinarian authorised by the Team Veterinarian inspected each female donor on each day that the oocytes were collected for this consignment and certified the donor to be free of clinical signs from contagious and infectious diseases.

- 6.3.4 Donors resided in the United States for at least 90 days prior to oocytes collection for this consignment.

6.4 Oocyte collection, processing and in vitro maturation

Note: *An oocyte collection is defined as oocytes collected during a single ovum pickup from a live donor.*

- 6.4.1 Only oocytes from the same female donor were washed and processed together.

- 6.4.2 All equipment/materials were disposed of and replaced with new items, or sterilised or disinfected in accordance with the current IETS Manual, before use and between different donors.
- 6.4.3 No oocytes of a lesser health status were processed within the laboratory at the same time as the germplasm for this consignment.
- 6.4.4 Any biological product of animal origin, including media constituents, used in oocyte recovery, maturation, washing and storage presented no animal disease risk. Media were sterilised prior to use by approved methods in accordance with the current IETS Manual and handled in such a manner as to ensure that sterility is maintained. Antibiotics were added to all fluids and media as recommended in the current IETS Manual.

6.5 Transport of oocytes from oocyte collection centre

- 6.5.1 The oocytes were processed, stored and transported to the in vitro produced embryo processing laboratory in a hygienic manner in accordance with recommendations of the current IETS Manual.
- 6.5.2 Only oocytes from the same individual donor were stored together in the same ampoule, vial or straw.
- 6.5.3 Ampoules, vials or straws were capped or sealed before transport.
- 6.5.4 Where a third party was used for transport, the storage container was sealed at the oocyte collection centre by the Team Veterinarian or an approved veterinarian who is a member of the embryo production team and the seal was not broken until receipt by the Team Veterinarian or a member of the embryo production team at the in vitro produced embryo processing laboratory.

6.6 Semen donors

- 6.6.1 The semen donor must be resident in the United States for 90 days prior to the collection of semen used to fertilise the oocytes in this consignment.

6.7 Semen

- 6.7.1 Only semen certifiable for export to Australia was used to fertilise the oocytes. Evidence was provided by the Team Veterinarian to USDA–APHIS for review.
- 6.7.2 If the semen was collected in another country, the semen importer provided a copy of certification from the country of origin to USDA–APHIS as evidence that the semen met Australian import requirements.

6.8 In vitro produced embryo processing laboratory

Note: *The in vitro produced embryo processing laboratory is the facility at which the in vitro produced embryos were processed through, at minimum, in vitro fertilisation, in vitro culture, embryo washing and freezing.*

- 6.8.1 This facility:
 - i) was on a property not subject to any restriction or quarantine measure with respect to contagious or infectious animal disease
 - ii) was under the supervision of the Team Veterinarian

iii) is a permanent structure that was built and maintained in accordance with the recommendations of the current IETS Manual

iv) was subjected to inspection at least once a year by USDA-APHIS.

6.9 Production and storage of embryos

6.9.1 During the production of embryos for export to Australia and prior to their storage, no oocytes or embryos of a lesser health status were processed at the same time using the same equipment and materials.

6.9.2 All equipment/materials were disposed of and replaced with new items, or disinfected in accordance with the recommendations of the current IETS Manual between different donors.

6.9.3 Any biological product of animal origin, including co-culture cells and media constituents, used in fertilisation, culture, washing and storage presented no animal disease risk. Media were sterilised prior to use by approved methods in accordance with the current IETS Manual and handled in such a manner as to ensure that sterility is maintained. Antibiotics were added to all fluids and media as recommended in the current IETS Manual.

6.9.4 Cleaning, and sterilisation or disinfection of, equipment were carried out in accordance with the recommendations of the current IETS Manual.

6.10 Embryos

6.10.1 The embryos were handled in accordance with the current IETS Manual:

i) All embryos are identified and can be traced to the male and female donors.

ii) Only embryos from the same female donor were washed together, and no more than ten embryos were washed at any one time.

iii) The zona pellucida of each embryo, before washing, was examined over its entire surface area at not less than 50X magnification to ensure that it is intact and free of adherent material.

iv) The embryos were washed at least ten times with at least 100-fold dilutions between each wash, and a new sterile micropipette was used for transferring the embryos through each wash.

v) The standard washing procedure includes additional washes with the enzyme trypsin.

vi) If performed, micromanipulation for biopsy for genetic testing was carried out only on embryos with intact zona pellucida after the standard washing procedure, and in suitable laminar-flow facilities which were properly cleaned and disinfected between batches.

6.11 Diagnostic testing

6.11.1 The samples were collected by an USDA accredited veterinarian.

6.11.2 Tests for disease were carried out at a laboratory approved by the competent authority to perform the required test.

6.11.3 The tests were conducted in accordance with the current OIE Manual.

- 6.11.4 The test reports provided to USDA to support certification must display the dates of sampling for the tests required, the type of test used and the test results. This information must be contained in a table against donor information, annexed to the health certificate, and verified and certified correct by the USDA-APHIS certifying officer.

6.12 Disease freedom

- 6.12.1 At the time of, and for 30 days after, each oocyte collection for this consignment, the United States was officially recognised by the OIE as a:

- i) foot and mouth disease free country where vaccination is not practised
- ii) contagious bovine pleuropneumonia free country

AND meets the current OIE Code Article definitions for country freedom from:

- iii) lumpy skin disease
- iv) Rift Valley fever
- v) brucellosis due to *B. melitensis*

AND was recognised by the Australian Government as a country free from foot and mouth disease where vaccination is not practised.

6.13 Vesicular stomatitis

- 6.13.1 During the 30 days prior to, and at the time of, each collection of oocytes, there was no clinical signs or reports of vesicular stomatitis at the premises where donor cows were kept and at the oocyte collection facility.

6.14 Bovine brucellosis

- 6.14.1 During the 30 days prior to, and at the time of, each collection of oocytes, the donor cows were from a herd that was located in a country or zone free from infection with bovine brucellosis (*B. abortus*) in accordance with the current OIE Code.
(Note: this includes donor cows from a herd that was located in a State or area officially free from infection with bovine brucellosis (*B. abortus*) and the herd was recognized as officially free of brucellosis by USDA-APHIS in accordance with the USDA Brucellosis Eradication Program.)

- 6.14.2 Embryos derived from oocytes were not collected within six months of vaccination of donors against brucellosis.

6.15 Bovine tuberculosis

- 6.15.1 During the 30 days prior to, and at the time of, each collection of oocytes, the donor cows were from a herd that was located in a country or zone free from bovine tuberculosis in accordance with the current OIE Code.
(Note: this includes donor cows from a herd that was located in a State or zone officially free from bovine tuberculosis and the herd was recognized as officially free of tuberculosis by USDA-APHIS in accordance with the USDA Tuberculosis Eradication Program.)

6.16 Bluetongue**6.16.1** Blood samples drawn from each donor:

- i) were subjected to a cELISA test to detect antibodies to the BTV group between 28 and 60 days after each collection of oocytes with negative results
- or
- ii) were subjected to an agent identification test on a blood sample taken on the day of collection with negative results.

6.17 Epizootic haemorrhagic disease (EHD)**6.17.1** Blood samples drawn from each donor:

- i) were subjected to the cELISA test to detect antibodies to the EHDV group between 28 and 60 days after each collection of the oocytes for this consignment with negative results
- or
- ii) were subjected to an agent identification test on a blood sample taken on the day of collection with negative result.

6.18 Infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV)**6.18.1** For all oocytes collected from the donors:

- i) The donor was kept in a herd where all eligible animals including the donor were vaccinated against IBR/IPV with a vaccine approved by the USDA at least 30 days prior to collection of oocytes. The vaccine was administered as per manufacturer's instructions for vaccination and revaccination
- and
- ii) A vaginal mucus sample[#] was collected from the donor immediately prior to washing/disinfecting the vagina in preparation for oocyte collection and tested for bovine herpesvirus-1 by the qRT-PCR for bovine herpesvirus-1 with negative results.
[#]Double guarded culture swabs, appropriately prepared for testing samples, were used to hygienically collect samples of vaginal mucus from the donors.

6.19 Bovine viral diarrhoea virus (BVDV)**6.19.1** At the time of each collection of oocytes, each female donor gave a negative result to one of the following tests for BVDV:

- i) an antigen-capture ELISA on peripheral blood leucocytes
- or
- ii) a monoclonal immunoperoxidase or other virus isolation test on blood or serum.

AND

- 6.19.2 If vaccinated, the donors were kept in a herd where all eligible animals including the donors were vaccinated against both BVDV-1 and BVDV-2 with a vaccine approved by the USDA at least 30 days prior to collection of oocytes. The vaccine was administered as per manufacturer's instructions for vaccination and revaccination.

6.20 Schmallerberg viruses

- 6.20.1 No cases of disease caused by Schmallerberg virus has been detected or reported in the United States.

6.21 Storage and transport

- 6.21.1 From the time of embryo freezing until export, the in vitro produced embryos in this consignment were stored for at least 30 days:
- i) in sealed sterile containers (e.g. straws, ampoules or vials) and identified in a legible and non-erasable manner as specified in the current IETS Manual. Goblets and canes were also identified as specified in the current IETS Manual
 - ii) only with other bovine germplasm collected for export to Australia, or of equivalent health status
 - iii) in storage or shipping containers containing only new, unused liquid nitrogen
 - iv) in a secure place within an approved centre or laboratory
 - v) under the supervision of the Team Veterinarian

6.22 Shipping containers (liquid nitrogen shippers/tanks)

EITHER

- 6.22.1 The shipping container was new.

OR

- 6.22.2 Immediately prior to loading, the shipping container was emptied and inspected and any loose straws removed. The shipping container, including all surfaces in contact with the straws, ampoules or vials was then disinfected with one of the following disinfectants: 2% available chlorine (e.g. chlorine bleach), 2% Virkon or irradiated at 50 kGy.

Date of disinfection/ irradiation

Disinfectant used/ active ingredient

[The veterinary certificate must indicate the option that applies. For used shipping containers, the date of disinfection, the disinfectant used and its active chemical must be recorded on the health certificate.]

6.23 Official government seals

- 6.23.1 Under the supervision of an Official Government Veterinarian prior to export to Australia:

- i) the containers (e.g. straws, ampoules or vials) for reproductive material in this consignment were checked as being sealed

- ii) the identity of the reproductive material was checked prior to being placed into new, unused liquid nitrogen in a shipping container for export that was new or disinfected as specified in this veterinary certificate.
- 6.23.2 Only bovine reproductive material (that is, semen and/or in vivo derived embryos and/or in vitro-produced embryos) that met Australian import conditions can be transported in the same shipping container.
- 6.23.3 An official government seal was applied by an Official Government Veterinarian to the shipping container and the number or mark on the seal recorded on the certificate.

Shipping container official government seal number

Glossary

Term or abbreviation	Definition
Approved veterinarian	Veterinarian officially approved, accredited or registered by the Veterinary Authority
Bovine or cattle	Animals belonging to <i>Bos taurus</i> or <i>Bos indicus</i> breed or to <i>Bos taurus</i> and <i>Bos indicus</i> crossbreed.
Donor animals	Live female cattle from which the oocytes were collected.
Embryo production team (EPT)	<p>A group of competent technicians, including at least one veterinarian approved by the Veterinary Authority, to collect and process oocytes and the production and storage of in vitro produced embryos for export to Australia.</p> <p>Where oocytes collection and in vitro maturation are performed separately to the EPT performing the in vitro fertilisation and in vitro culture, there must be at least one veterinarian in each team.</p>
Freeze, freezing or frozen	Process involving cryopreservation of embryos using cryopreservatives and/or liquid nitrogen. The main techniques used for embryo cryopreservation are the rapid freeze (vitrification) or the slow programmable freezing.
In vitro produced embryos	Embryos produced in vitro involve the collection of oocytes from ovaries of live donors, in vitro maturation of oocytes in preparation for fertilisation, in vitro fertilisation, then in vitro culture to the morula/blastocyst stage at which they are ready for transfer to recipients or for freezing.
In vivo derived embryos	Embryos at the morula/blastocyst stage collected by flushing the uterus of super-ovulated donors
Micromanipulation	The process where in vivo derived or in vitro produced embryos have been subjected to biopsy for genetic testing. For this review, this does not include splitting, transgene injection, intracytoplasmic sperm injection, nuclear transfer or other interventions that breach the integrity of the zona pellucida.
Official government veterinarian	A veterinarian authorised by the Veterinary Authority of the country to perform certain designated official tasks associated with animal health and/or public health and inspections of commodities and, when appropriate, to certify in accordance with OIE Code Chapters 5.1. and 5.2.
Oocyte collection facility	Premises consisting of an oocyte recovery area and a permanent or mobile laboratory for the processing of oocytes and in vitro maturation before transporting to the permanent processing laboratory.
Permanent in vitro produced embryo processing laboratory	Laboratories where the matured oocytes are processed further and in vitro fertilisation, in vitro culture and freezing of in vitro produced embryos are performed.
Semen donors	Mature male cattle from which semen was collected, processed and stored and which met the health conditions specified in the veterinary certificate.
Storage facility	Facilities where frozen bovine in vitro produced embryos are stored until export.
Team Veterinarian	A veterinarian officially approved, accredited or registered by the Veterinary Authority responsible for supervising the EPT. The EPT veterinarian is responsible for all team operations which include the hygienic collection of oocytes and all other procedures involved in the production of embryos for export to Australia.
Veterinary Authority	The governmental authority of an OIE member, comprising veterinarians, other professionals and para-professionals, having the responsibility and competence for ensuring or supervising the implementation of animal health and welfare measures, international veterinary certification and other standards and recommendations in the OIE Code in the whole territory.

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