Attachment B





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

FINAL REPORT

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AUSTRALIA

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EXECUTIVE SUMMARY

In this import risk analysis report (IRA) AQIS has assessed the risk of importing disease agents (other than scrapie infective agent) with ovine and caprine genetic material from the United States of America (USA), Canada and the Member States of the European Union (EU). Australia currently prohibits the importation of ovine and caprine semen from all countries except New Zealand (NZ) and ovine and caprine embryos from all countries except NZ and the Republic of South Africa (RSA). Australia accepts that both NZ and RSA are free from scrapie. The risks of scrapie entry were assessed separately and the final report released with this document. The final conditions for importation of ovine and caprine genetic material from Canada, the USA and EU are the amalgamation of the risk management options identified by these two risk analyses.

The hazards identified in this import risk analysis (IRA) are ovine and caprine disease agents which could be introduced with ovine and caprine embryos and/or semen and adversely affect Australian livestock industries, other animal based industries and/or native species.

The risks are qualitatively assessed. The assessment includes:

- consideration of the epidemiological features affecting the likelihood of pathogens infecting or contaminating ovine and caprine semen and embryos;
- the likelihood of pathogens remaining after the semen is prepared and embryos washed; and
- the likelihood of infected or contaminated semen or embryos causing disease in recipients or offspring and this disease then spreading to other susceptible hosts.

The following pathogens were identified as requiring risk management:

- foot and mouth disease virus,
- bluetongue virus,
- capripoxvirus (sheep and goat poxvirus),
- Mycobacterium (avium subsp) paratuberculosis,
- Brucella melitensis,
- Brucella ovis,
- Mycoplasma capricolum subsp. capripneumoniae,
- Mycoplasma agalactiae,
- Chlamydia psittaci (enzootic abortion of ewes),
- maedi-visna virus,
- caprine arthritis encephalitis virus and
- jaagsiekte virus.

Risk management options for the importation of genetic material were evaluated in terms of their efficacy at reducing the assessed risk and also the level of restriction that their adoption would place on trade. Initially, OIE recommendations were considered and are included in the final conditions if considered suitable. Where it was considered that necessary risk management was not adequately addressed by OIE recommendations alternative measures are required.

Quarantine measures include country, zone or flock freedom, testing of donor animals, treatment of semen and washing embryos.

Final conditions for importation are attached.

ABBREVIATIONS AND ACRONYMS

AGID	agar gel immunodiffusion (test)
AI	artificial insemination
APHIS	Animal and Plant Health Inspection Service of the USDA
AQIS	Australian Quarantine and Inspection Service
AQPM	Animal Quarantine Policy Memorandum
AUSVETPLAN	Australian Veterinary Emergency Plan
BTV	bluetongue virus
CA	contagious agalactia
CAE	caprine arthritis/encephalitis
CCPP	contagious caprine pleuropneumonia
CEE	central European encephalitis
CFT	complement fixation test
CPV	capripox virus
ELISA	enzyme-linked immunosorbent assay
ET	embryo transfer
EU	Member States of the European Union
FMD	foot and mouth disease
HAI	haemagglutination inhibition (test)
IETS	International Embryo Transfer Society
IRA	import risk analysis
LI	louping ill
LIV	louping ill virus
LIRV	louping ill and related viruses
OJD	ovine Johne's disease
JV	jaagsiekte virus
MV	maedi-visna
OIE	Office International des Epizooties
PAQ	post-arrival quarantine
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEQ	pre-export quarantine
RSSE	Russian spring-summer encephalitis
SSEV	Spanish sheep encephalitis virus
TBE	tick-borne encephalitis (caused by members of the flavivirus group)
TSEV	Turkish sheep encephalitis virus
USA	United States of America
USDA	United States Department of Agriculture
VNT	virus neutralisation test
ZP	zona pellucida

GLOSSARY

Accredited artificial insemination (AI) centre

A facility for the collection, handling and storage of semen accredited by the *Veterinary Administration* and used exclusively for donor animals which meet the conditions set out in *Code* (Article 4.2.2.2.).

Centre veterinarian

The centre veterinarian directly supervises an *accredited AI centre* and is approved by the *Veterinary Administration*.

Code

Office International des Epizooties (OIE) International Animal Health Code.

Consequence assessment

The relationship between specified exposures to a biological agent and the consequences of those exposures. A causal process must exist by which exposures produce adverse health or environmental consequences, which may in turn lead to socio-economic consequences. The consequence assessment describes the potential consequences of a given exposure and estimates the probability of them occurring.

Embryo

For the purposes of this risk analysis the embryo is regarded as the conceptus from fertilised single cell to unhatched blastocyst stages (zona pellucida intact). (Technically, the period of the embryo is the stage during which the main tissues, organs and systems are formed).

Exposure assessment

The biological pathway(s) necessary for exposure of animals and humans in the *importing country* to the hazards (in this case the pathogenic agents) released from a given risk source, and estimating the probability of the exposure(s) occurring.

Genetic material

Embryos, ova and/or semen.

Incubation period

The longest period which elapses between the introduction of the pathogen into the animal and the occurrence of the first clinical signs of the disease.

Intrauterine transmission

Transmission of infection from infected dam to embryo or foetus in the uterus.

IETS Manual

Manual of the International Embryo Transfer Society (IETS), 3rd Edition, April 1998. Edited by Stringfellow DA and Seidel SM. Published by: IETS, 1111 North Dunlap Ave, Savoy, Illinois, 61874 USA.

in vitro

A process or procedure performed outside the body in a test tube or other laboratory apparatus.

in vivo

A process occurring in a living organism or under natural circumstances.

Manual

OIE Manual of Standards for Diagnostic Tests and Vaccines.

National control program

A program that is nationally consistent and uses flock accreditation based on monitoring and testing. The program is usually supported by a government/industry compensation scheme.

Pre-collection period

The pre-collection period is 30 days immediately prior to the first collection of semen or embryos.

Release assessment

The biological pathway(s) necessary for an importation activity to "release" (that is, introduce) pathogenic agents into a particular environment, and estimating the probability of that complete process occurring, either qualitatively (in words) or quantitatively (as a numerical estimate). The release assessment describes the probability of the "release" of each of the potential hazards (the pathogenic agents) under each specified set of conditions with respect to amounts and timing, and how these might change as a result of various actions, events or measures.

Risk estimation

Consists of integrating the results from the release assessment, exposure assessment, and consequence assessment to produce overall measures of risks associated with the hazards identified at the outset. Thus risk estimation takes into account the whole of the risk pathway from hazard identified to unwanted outcome.

Stamping-out policy

Carrying out under the authority of the *Veterinary Administration*, on confirmation of a disease, the killing of the *animals* which are affected and those suspected of being affected in the herd and, where appropriate, those in other herds which have been exposed to infection by direct animal to animal contact, or by indirect contact of a kind likely to cause the transmission of the causal pathogen. All susceptible animals, vaccinated or unvaccinated, on an infected premises should be killed and their carcasses destroyed by burning or burial, or by any other method which will eliminate the spread of infection through the carcasses or products of the animals killed.

Team veterinarian

The team veterinarian supervises the embryo collection team, and is responsible for all team procedures and should be specifically approved for this purpose by the *Official Veterinarian*.

Washing embryos

The washing of *in-vivo* derived embryos with intact zona pellucida as described in Chapter 6 of the *IETS Manual* where embryos are washed ten times to remove pathogens.

Veterinary Administration

The National Veterinary Service having authority in the whole country for implementing and supervising or auditing the carrying out of the animal health measures and certification process which the *Code* recommends.

1. INTRODUCTION

1.1 Scope of risk analysis

This document analyses the risks, other than scrapie, associated with importing *in vivo* derived ovine and caprine embryos and ovine and caprine semen from the United States of America (USA), Canada and Member States of the European Union¹ (EU) into Australia. The analysis is confined to a consideration of quarantine risks, ie. the probability of entry and establishment of exotic disease agents. A number of ovine and caprine diseases that occur in USA, Canada and EU do not occur in Australia. Imported semen and embryos may transmit some of these disease agents to susceptible recipients or their offspring.

The IRA:

- identifies the disease hazards other than scrapie which may be found in semen and *in vivo* derived embryos and which have the potential to cause harm,
- assesses the probability of transmission of these disease agents by semen and embryos to other susceptible animals resulting in establishment and spread of disease,
- assesses the probability of adverse consequences of establishment of these disease agents,
- identifies the risk management options for minimising the risks of introducing diseases other than scrapie into Australia with ovine and caprine semen and embryos,
- recommends risk management measures which could be applied to each disease agent before importation, and
- provides final quarantine conditions for importation.

1.2 Current quarantine policy and practice

Current quarantine policy relating to ovine and caprine embryos and semen is described in the IRA for scrapie.

Australia permits the importation of *in vivo* derived ovine and caprine embryos from New Zealand (NZ) and the Republic of South Africa (RSA) and ovine and caprine semen from NZ. As the animal health status of the USA, Canada and the EU differs from NZ and RSA the development of conditions for importation from these countries required an IRA.

2. HAZARD IDENTIFICATION

AQIS has used a process of categorisation to identify disease agents requiring further consideration in this IRA. A disease agent is identified as a hazard and the risk of entry via embryos or semen is assessed in this paper if it is :

• exotic to Australia,² or present in Australia but subject to a *National control program* and

¹ The Member States of the EU are Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, the Netherlands, Portugal, Sweden, Spain and the UK.

² More virulent strains or serotypes may be reported overseas.

- the cause of significant disease and
- potentially transmitted by ovine and/or caprine genetic material and
- reported in at least one of the countries which are the subject of this IRA.

Disease agents that have been identified as requiring risk assessment are grouped according to the *Code* listing in Table 1. Disease agents associated with disease in sheep and goats and not identified as hazards for the purposes of this IRA are listed in appendix 1.

3. EXPOSURE PATHWAYS

Exposure pathways trace the potential route/s by which animals and humans in Australia may be exposed to pathogens harboured by imported genetic material. For an exotic disease agent to become established and spread in sheep or goats (or other susceptible species) in Australia through the importation of genetic material the pathogen must:

- be associated with ovine and/or caprine genetic material,
- survive exposure to environmental stressors, eg. washing, exposure to diluents, antibiotics, freezing, etc. and remain infectious after importation and transfer or insemination,
- infect the recipient of the genetic material or offspring, and
- spread from the index case(s) to a sufficient number of susceptible hosts to become established.

Association of the disease agent with imported genetic material depends on several factors which may differ for embryos and semen. Infection of embryos depends on the susceptibility of the sheep or goat to the disease agent and whether the tissue tropism includes the ovaries and other parts of the genital tract. Transmission by embryo transfer (ET) may also arise if the disease agent is a contaminant of the embryo storage medium or is present on contaminated personnel, instruments or equipment.

Few studies have been made of the interaction between embryos and pathogens in small ruminants in comparison with those conducted on bovine embryos. As a consequence, few disease agents affecting sheep and goats have been categorised by the International Embryo Transfer Society Import/Export Committee (IETS) Research subcommittee for their capacity to be transmitted via ET. The role of the zona pellucida (ZP) in preventing penetration of infectious agents into the embryo and also as a medium for the carriage of pathogens is discussed in the draft risk analysis for bovine semen and embryos from Argentina and Brazil (AQPM 1999/34). However, characteristics of embryos and their interactions with pathogens cannot be generalised. Embryos of different species differ in the glycoprotein composition of the ZP. This structure in sheep and goats differs from that in cattle (Chen and Wrathall 1989; Dunbar *et al* 1991). It has been suggested that ovine ZP is 'stickier' than that of bovine embryos, and less likely to resist penetration and adherence of pathogens (Singh *et al* 1997). This may explain the higher probability of binding between the ZP and various pathogens in these species. In the absence of relevant information, infection patterns for sheep and goat embryos and semen are based on studies of infection of bovine genetic material. Nevertheless, the uncertainty of this extrapolation is acknowledged.

Semen is a complex association of cells and fluids from several organs. Even though some disease agents have been reported in the semen of rams, most are found in the seminal fluid or leucocytes rather than within or attached to the spermatozoon. Some pathogens, eg. certain RNA viruses such

as bluetongue virus (BTV), have been implicated in gamete infections (Eaglesome *et al* 1980), but it is generally accepted that this mode of infection is not significant. Increased white cell numbers due to accompanying infection can increase the likelihood of the presence of some pathogens in semen (de la Concha-Bernejillo *et al* 1996). Similarly blood may be occasionally present depending on the collection technique used or if concurrent infections cause damage to capillaries resulting in infected blood cells in semen, eg. BTV.

Without the imposition of risk management measures, the practice of collection, handling, transport and transfer of embryos or semen to recipients represents a very direct exposure pathway for any disease agents present in imported material. In Australia sheep and goats are usually kept in extensively managed flocks providing an opportunity for the direct spread of disease from infected animals to other stock or other animals in the surrounding environment.

Disease agents considered in this IRA³ (Geographic distribution according to OIE reports) Table 1

				Т	
Hazard	Susceptible Species	Australia Health Status	US Health Status	Canada Health Status	Member States of the EU
OIE List A	• •			-	
Foot and mouth disease virus	All cloven hoofed animals	Free (1871)	Free (1929)	Free (1952)	FREE; Austria (1981), Belgium (1976), Denmark (1983), Finland (1959), France (1981), Germany (1988), Greece (1996), Ireland (1941), Italy (1993), Luxembourg (1964), Netherlands (1984), Portugal (1984), Spain (1986), Sweden (1966), UK (1981)
Bluetongue virus	Cattle, goats, sheep	Confined to certain regions	Confined to certain regions	Confined to one region	NEVER REPORTED; Austria, Belgium, Denmark, Finland, France, Germany, Ireland, Italy, Luxembourg, Netherlands, Sweden, UK FREE; Portugal (1959), Spain (1960) LOW SPORADIC OCCURRENCE; Greece ⁴
Capripox virus	Sheep, cattle, goats, humans	Never reported	Never reported	Never reported	NEVER REPORTED; Luxembourg, Finland FREE: Austria (1954); Denmark (1879), France (1964), Germany (1920), Ireland (1850), Italy (1983), Netherlands (1893), Portugal (1970), Spain (1968), Sweden (1934), UK (1856), Greece (1998)
OIE List B					
Mycobacterium (avium subsp.) paratuberculosis (Johne's disease)	Cattle, sheep, goats, camelids	Enzootic in certain regions National control program	Low sporadic occurrence	Low sporadic occurrence	FREE; Italy (1995), Finland (1996) EXCEPTIONAL OCCURRENCE; Belgium LOW SPORADIC OCCURRENCE; Austria, France, Germany, Greece, Ireland, Denmark, Luxembourg, Netherlands, Portugal, Sweden, Spain, UK
Brucella ovis	Sheep	Low sporadic occurrence	Low sporadic occurrence	Low sporadic occurrence	NEVER REPORTED; Finland, Denmark, Ireland, Netherlands, Sweden, UK FREE; Germany (1986) NOT REPORTED; Belgium, Luxembourg, Portugal LOW SPORADIC OCCURRENCE; Austria, France, Spain NO INFORMATION AVAILABLE; Greece, Italy
Brucella melitensis	Sheep, goats, cattle, pigs, humans (wildlife species)	Not reported ⁵	Not reported (1972)	Never reported	NEVER REPORTED; Finland, Ireland, Denmark, Luxembourg, Netherlands, Sweden, UK LAST REPORTED OCCURRENCE; Germany (1992) NOT REPORTED ⁶ ; Austria, Belgium LOW SPORADIC OCCURRENCE; Greece, Italy, Portugal, Spain ENZOOTIC; France

³ Figures in brackets indicate the year of last occurrence for countries officially free (when known) ⁴ Confined to certain regions

Hazard	Susceptible Species	Australia Health Status	US Health Status	Canada Health Status	Member States of the EU
Mycoplasma capricolum subsp. capripneumoniae	Goats	Never reported	Never reported	Never reported	LAST REPORTED OCCURRENCE; Sweden (1983) NEVER REPORTED; Belgium, Finland, France, Germany, UK Ireland, Denmark, Luxembourg,
(Contagious caprine					Netherlands
pleuropneumonia)					NOT REPORTED; Austria, Italy, Portugal, Spain
					LOW SPORADIC OCCURRENCE; Greece
Maedi-visna virus	Sheep	Never reported	Never reported ⁷	Low sporadic	LAST REPORTED OCCURRENCE; Ireland (1986)
				occurrence	NOT REPORTED; Austria, Italy ⁸
					EXCEPTIONAL OCCURRENCE; Finland
					LOW SPORADIC OCCURRENCE; Belgium, Germany, Greece, Denmark, Luxembourg,
					Netherlands, Portugal, Spain, Sweden, UK
Caprine	Goats	Low sporadic	Low sporadic	Low sporadic	NEVER REPORTED; Luxembourg, Finland, Netherlands, Portugal, Spain ¹⁰
arthritis/encephalitis virus		occurrence ⁹	occurrence	occurrence	NOT REPORTED; Austria, Belgium
					SUSPECTED BUT NOT CONFIRMED; Denmark, Ireland,
					LOW SPORADIC OCCURRENCE; Germany, Sweden, UK, Greece, Italy
		11	12		ENZOOTIC; France
Mycoplasma agalactiae	Sheep, goat	Not reported ¹¹	Never reported ¹²	Never reported	NEVER REPORTED; Finland, Germany, Ireland, Luxembourg, Netherlands, Sweden
(Contagious agalactia)					NOT REPORTED; Belgium, Denmark, UK
					EXCEPTIONAL OCCURRENCE; Spain
					LOW SPORADIC OCCURRENCE; France, Greece, Italy, Portugal,
		12			SUSPECTED BUT NOT CONFIRMED; Austria
Leptospira spp.	All mammals	Present ¹⁵	Present	Present	PRESENT; Sweden, Austria, Italy, Spain, France, Germany, Greece, Ireland, Netherlands,
	(including				Portugal, UK
	humans)				LAST REPORTED OCCURRENCE; Finland (1997)
					NOT REPORTED; Denmark, Luxembourg, Belgium

⁵ Brucella melitensis is reported sporadically in humans in Australia. The disease is described as an uncommon but serious infection, affecting people who have aquired the infection overseas (Chan and Hardiman 1993). This is probably also true of the USA, Canada and Member States of the EU.

⁶ Not reported in this table means that the disease is probably present in the country but has not been reported to the OIE or described in the scientific literature

⁷ There are numerous reports of MVV in USA as ovine progressive pneumonia (OPP), Montana sheep disease or ovine lentivirus infection (Brodie *et al* 1994) and Snowder *et al* (1990) writes that it is well established that OPP is wide-spread in sheep in North America.

⁸ Infection appears to be widespread in France and Italy (Lujan et al 1993).

⁹ CAEV is included as a hazard in this IRA even though the virus is present in Australia. There is evidence that strains of CAE which can cause disease in sheep are not present in Australia but are apparently present overseas (Smith *et al* 1985).

¹⁰ Contreras *et al* (1998) report CAE in goats in Spain

¹¹ Mycoplasma agalactiae has been isolated in Australia, but the Australian strains do not produce contagious agalactia in sheep.

¹² *M* agalactiae capable of causing mastitis in goats is now considered to be present in the USA (Kinde et al 1994)

¹³ Pathogenic leptospire serovars present in sheep or goats in the EU, Canada and the USA may not be present in Australia.

Hazard	Susceptible Species	Australia Health Status	US Health Status	Canada Health Status	Member States of the EU
<i>Chlamydia psittaci</i> Enzootic abortion of ewes	Sheep, goats	Not reported ¹⁴	Low sporadic occurrence	Low sporadic occurrence	NEVER REPORTED; Finland, Denmark, Luxembourg Sweden NOT REPORTED; Austria, Belgium, Italy, EXCEPTIONAL OCCURRENCE; Spain LOW SPORADIC OCCURRENCE; France, Germany, Greece, Ireland, Netherlands, Portugal, UK
Jaagsiekte virus	Sheep and goat	Never reported	Never reported ¹⁵	Low sporadic occurrence	NEVER REPORTED; Finland Luxembourg, Sweden NOT REPORTED; Austria, Portugal, Spain ¹⁶ Belgium, Germany, Italy, Netherlands LOW SPORADIC OCCURRENCE; France, Greece, Ireland, Denmark, UK ¹⁷
Other disease agents					·
Louping-ill and related viruses	Sheep, goats , humans (and a wide range of vertebrates)	Never reported	Not reported	Not reported	Reported in most of Europe, including Spain, Greece and UK

¹⁴ The strains of *Chlamydia psittaci* that cause typical enzootic abortion have not been reported in Australia.
¹⁵ Other reports suggest that jaagsiekte is present in the USA (Cutlip and Young 1982).
¹⁶ Lujan *et al* (1993) report jaasiekte infection of sheep in Spain.
¹⁷ Other reports indicate a high prevalence in the UK (Hunter and Munro 1983; Houwers and Terpestra 1984)

4. RISK ASSESSMENT

In assessing the level of risk associated with the importation of genetic material, AQIS considers not only the differences in disease status between exporting and importing countries, but also the pathogenesis of the diseases of concern and relevant epidemiological attributes of the agent, host and environment.

The *Code* recommends that risk assessments are separated into three steps; release, exposure and consequence assessments. In this paper, the release and exposure assessments are combined and estimated as the likelihood of entry, establishment and spread.

The potential for association of pathogens with embryos and accompanying fluids depends largely, but not entirely, on the tissue tropism of the pathogen. Clear evidence of this potential association is provided if the agent is detected in the embryo or associated embryonic fluids or if there is evidence of disease transmission via natural breeding or ET. There are other patterns of agent distribution which have the potential for infection of embryos or embryonic fluid. In this risk assessment it is assumed that if there is evidence of vertical transmission it follows that the agent may colonise the internal reproductive tract. Further, it is assumed that if the agent has been recovered from any part of the reproductive tract there is the potential for contamination of fluids associated with embryos. This is because the tract is a more or less continuous tube. If the disease is characterised by a blood-borne phase, ie. a viraemia or bacteraemia, it is assumed that any part of the body including the embryonic fluids could be exposed to the agent. Contamination of embryos during viraemic/bacteraemic periods could also occur through direct exposure to blood. Embryo collection is usually a surgical procedure and it is virtually impossible to obtain blood free flushing fluids when embryos are collected by this means (Singh *et al* 1997).

Clear evidence that disease agents are present in semen exists if the agent is detected in semen, or venereal and/or AI transmission has been reported.

Estimates are provided of the probability of entry, establishment and spread (release and exposure assessments) of each of the pathogens considered in this IRA paper through the unrestricted importation and transfer of genetic material, ie. without risk management measures. Because of the level of uncertainty associated with these estimates, the descriptors of the level of risk have been limited to four, viz. negligible, low, moderate and high, rather than using an expanded range (slight, extreme, etc.) which would imply a greater level of certainty than is currently valid.

The probability of entry, establishment and spread is estimated to be:

- **Negligible** for those disease agents:
 - not generally present in the genital tract, eg. found only in unassociated tissues, and
 - viraemia or bacteraemia does not generally occur during infection or is generally of short duration, and
 - for which there is no evidence of venereal transmission or transmission by AI or ET.
- **Low** for those disease agents:
 - either
 - not generally present in the genital tract, and
 - which cause bacteraemia or viraemia, and
 - for which there is no evidence of venereal transmission or transmission by ET or AI

- which are transmitted by vector.
- **Moderate** for those disease agents :

either

- not generally associated with the genital tract, and
- which have been isolated from embryos/semen, and
- for which there is no evidence of venereal transmission, and
- which spread readily via direct contact.

or

- generally present in the genital tract, and
- for which there is no evidence of transmission by ET or AI, and
- which spread readily via direct contact.
- **High** for those disease agents
 - for which there is evidence of transmission by ET or AI, and
 - which spread readily via direct contact.

The assessment of the consequences of disease establishment in Australia combines an economic and environmental assessment. These separate assessments are combined according to the following matrix.

Probability of	HIGH	low	high	high	high	
adverse	MODERATE	low	moderate	moderate	high	
environmental	LOW	low	low	moderate	high	
consequences	NEGLIGIBLE	negligible	low	low	low	
		NEGLIGIBLE	LOW	MODERATE	HIGH	
		Probability of adverse economic consequences				

A risk estimate (estimate of the integrated risk) for each disease agent was obtained by combining the risk ratings for the probability of agent entry, establishment and spread with the probability of adverse consequences (release assessment, exposure assessment and consequence assessment). This is an estimated probability that a disease agent establishes in susceptible animals in this country and results in significant harm in human, economic and environmental terms. The integrated risk estimate for each disease agent is derived from the following matrix:

Probability of	NEGLIGIBLE	low	negligible	negligible	negligible
agent, entry,	LOW	moderate	low	low	negligible
establishment	MODERATE	high	moderate	low	negligible
and spread	HIGH	high	high	moderate	low
		HIGH	MODERATE	LOW	NEGLIGIBLE
		Probab	oility of adverse con	isequences	

In this risk analysis, risk management measures are considered unnecessary for disease agents with a negligible rating.

4.1 Foot and mouth disease virus

Sheep are highly susceptible to infection with foot and mouth disease virus (FMDV). Both sheep and goats have been considered to play an important role in the epidemiology of foot and mouth disease in some European and Turkish outbreaks (Pay 1988). In sheep and goats the disease is usually mild or inapparent

but expression depends on the strain of virus, the breed of the animal and environmental conditions. Infected animals usually remain asymptomatic while excreting foot and mouth disease virus (FMDV) and may carry the virus for up to over 7 months (Sharma 1978). The incubation period after natural exposure is from 2 to 8 days (Mann and Sellers 1990).

Evidence that FMDV infects ovine and caprine embryos and semen

The presence of FMDV has been reported in bovine genetic materials but not in that of sheep or goats. Even so, FMDV is widely distributed in the tissues of infected animals, including vaginal tissues (Srinivas and Rajasekhar 1992) and blood of infected sheep (Sharma and Murty 1981). FMDV has been recovered from embryo collection fluid taken from experimentally infected donor cows. FMDV can spread via semen (Mann and Sellers 1990) but transmission of infection via semen has not been reported in sheep or goats. Infected bovine semen has been shown to transmit virus to other animals which suggests that this route of transmission may be possible in sheep and goats.

Probability of FMDV entry, establishment and spread

FMDV is not typically associated with the genital tract but has been detected in semen and can survive freezing (Bane 1981). Sheep and goats excrete FMDV for long periods even when asymptomatic (Sharma and Murty 1981). In addition, interspecies transmission has occurred during several outbreaks. In the Sikkim outbreak goats transferred the disease to cattle and in the UK, FMD spread rapidly from sheep to cattle (Srinivas and Rajasekhar 1992). FMD is very contagious, and infected sheep and goats are likely to be involved in the spread to other cloven hooved animals. Consequently, the probability that genetic material collected from infected sheep or goats results in the entry, establishment and spread is estimated to be moderate for embryos and semen.

Probability of adverse consequences arising through FMDV entry, establishment and spread

Ruminants and pigs are susceptible to FMDV. Losses would result due to deaths, morbidity, cost of eradication and/or control measures and the effect on trade. The social and economic effects of the spread of FMD in this country are detailed in the AUSVETPLAN (1996). The probability of adverse economic consequences is estimated to be high. The effect of FMDV on native Australian animals is not fully known but birds, reptiles and amphibians are not susceptible. Snowdon (1968) failed to infect various Australian mammals including 3 species of kangaroo, a wombat, a wallaby, a potoroo, a bandicoot, a possum, an echidna, a dasyurid and a water rat. Consequently the probability of adverse environmental consequences is estimated to be low. The overall estimate for probability of adverse consequences is high.

Risk estimate for FMDV

The risk estimate for FMDV without risk management measures is moderate for embryos and high for semen.

4.2 Bluetongue virus

BTV is transmitted by a range of *Culicoides* species. Serotypes 4 and 10 have caused epidemics in parts of Europe and serotypes 2,10,11,13 and 17 are endemic in North America (Gibbs and Greiner 1994). These serotypes are exotic and not amongst the eight serotypes of BTV which infect livestock in parts of Australia. The virulence of BTV serotypes varies considerably and Australian serotypes are considered to be less virulent than some overseas serotypes (Geering *et al* 1995).

Although BTV may infect many species of ruminant, sheep are usually the most severely affected. Viraemia in sheep and goats commences from 3 days post infection and may last up to 54 days (Koumbati *et al* 1999). The *Code* recognises an infective period for BT of 60 days. Sheep breeds from temperate

countries are more susceptible to BT than tropical breeds and the disease is now considered to be generally a disease of temperate areas. For example, in temperate areas such as California BT outbreaks often occur annually causing significant economic impact (Gibbs and Greiner 1994).

Evidence that BTV infects ovine and caprine embryos and semen

Reproductive disorders including early embryonic deaths, abortions, malformed foetal lambs, transient infertility in rams, and shedding of virus in semen have been described as BT related (Osburn 1994). BTV may be transmitted transplacentally but this occurs infrequently (Mellor and Boorman 1995). The transmission of BTV with incompletely washed embryos from infected to susceptible sheep has been described (Gilbert *et al* 1987). Ovine embryos exposed *in vitro* to BTV remained infected after 10 washes, however the integrity of embryos and duration of exposure to BTV during this study was unclear (Singh *et al* 1997).

BTV is shed in the semen of infected rams (Hare *et al* 1988). However, Hare *et al* (1988) found that ewes bred to these rams did not become infected.

Probability of BTV entry, establishment and spread

BTV has been transmitted by infected ovine embryos. The virus is shed in ovine semen but there is no clear evidence that infection is transmitted by naturally infected ovine semen. Whilst studies in cattle suggest that this form of transmission can occur transmission of BTV by ovine genetic material is considered infrequent (Mellor and Boorman 1999) and unimportant in the epidemiology of the disease (Geering *et al* 1995). Consequently, the probability that BTV entry could occur through infection of recipients of infected embryos or semen is estimated to be low.

The influence of vectors on the viruses they transmit is poorly understood and Tabachnick *et al* (1991) suggest that vector/serotype specificity has not been demonstrated. However, Australian studies have shown that the capacity of *Culicoides* species feeding on sheep to transmit BTV (vector competence) varies with serotype (Standfast *et al* 1985). Also, when exotic serotypes are introduced into different ecosystems they appear to die out if efficient vectors are absent (Gibbs and Greiner 1994). The vector competence of Australian *Culicoides* species for exotic strains of BTV is unknown, but based on overseas experience, it is estimated that even if recipients or progeny became infected via imported embryos or semen, the probability of spread to other ruminants is unlikely. Consequently, the probability of BTV entry, establishment and spread in the Australian zone of possible BT transmission is estimated to be low in the absence of risk management measures.

Probability of adverse consequences arising through BTV entry, establishment and spread

All domestic ruminants are susceptible to BTV but only sheep and deer show clinical symptoms. A detailed assessment of the social and economic effects of the spread of exotic serotypes of BTV is presented in AUSVETPLAN (1996). The probability of adverse economic consequences is estimated to be moderate. The effect of BTV on native Australian animals is unknown but there are no reports of BT in mammals other than ruminants, birds, reptiles or amphibians. Consequently the probability of adverse environmental consequences is estimated to be low. The overall estimate for probability of adverse consequences is moderate.

Risk estimate for BTV

The risk estimate for BTV without risk management measures is low for embryos and semen.

4.3 Capripoxvirus (sheep and goat pox virus)

Apart from strain differences, expressed as host preferences for either sheep or goats, pox viruses recovered from infected sheep and goats are considered to be indistinguishable (Kitching and Taylor 1985; Munz and Dumbell 1994) and are referred to by the *Code* as capripoxvirus (CPV).

Capripox is endemic in most of Africa, the Middle East and Asia. The disease was present in Greece until 1996. In 1998 Greece reported to the OIE that the disease was eradicated by a stamping out/non vaccination policy and previously affected areas were screened serologically.

Mortality rates may reach 10% in endemic areas and 100% in imported animals. Morbidity rates can reach 90% in sheep, but less in goats (*Code*; Munz and Dumbell 1994). Incubation periods range from 2 days with experimental infection (Merza and Mushi 1990) to 3 weeks in natural infections but 12 days is accepted as the average (Geering *et al* 1995; *Code*). Susceptibility to disease is breed dependent in sheep and infected resistant breeds show few signs of disease. The disease lasts from 3 to 4 weeks but does not become chronic (Geering *et al* 1995).

Evidence that CPV infects ovine and caprine embryos and semen

Specific information is not available on which to base an evaluation of the potential for transmission of capripoxviruses by ovine and caprine embryos. Viraemia occurs and may result in exposure of embryos to infection. Intrauterine transmission can occur in the course of generalised cowpox infection (Mayr and Czerny 1990).

No information is available on the transmission of capripoxvirus in semen. Orchitis is reported in goats (Merza and Mushi 1990) and in both sheep and goats pox lesions may occur on the skin and mucous membrane surfaces, including the prepuce. These symptoms may result in viral contamination of semen before or during collection from infected animals. Transmission via semen is considered likely (AUSVETPLAN 1996).

Probability of CPV entry, establishment and spread

In the absence of information to the contrary, and extrapolating from transmission of other poxviruses, it is assumed that there is a risk that infected genetic material may be collected from infected animals. Capripox is a contagious disease and rapid spread can occur within large flocks (Kitching and Taylor 1985) and via fomites and insect vectors (Merza and Mushi 1990). Because of the high mortality rate in susceptible populations, ease of diagnosis and lack of a carrier state, it is highly likely that CPV would be quickly eradicated if introduced into Australia. Based on this limited information, it is assumed that the probability of entry, establishment and spread is low.

Probability of adverse consequences arising through CPV entry, establishment and spread

Sheep and goats are susceptible to CPV and Merinos and British breeds are very susceptible to disease (AUSVETPLAN 1996). Losses would result from death of sheep, morbidity, the cost of eradication and/or control measures and some effect on trade, especially the potential loss of wool markets (AUSVETPLAN 1996). Based on this the probability of adverse economic consequences is estimated to be high. The effect of CPV on native Australian animals is unknown but there are no reports of CP in other mammals, birds, reptiles, amphibians, marsupials or monotremes. Consequently the probability of adverse environmental consequences is estimated to be low. The overall estimate for probability of adverse consequences is moderate.

Risk estimate for CPV

The risk estimate for CPV without risk management measures is low for embryos and semen.

4.4 Mycobacterium paratuberculosis (Johne's disease/paratuberculosis)

Isolates of *M paratuberculosis* (*M avium* subsp. *paratuberculosis*) appear to demonstrate host preferences rather than host specificities (Gunnarson and Fodstad 1979; Huchzermeyer *et al* 1994). Sheep strains usually only infect sheep, causing ovine Johne's disease (OJD) and characteristically can be extremely difficult to culture (Whittington *et al* 1999). Nevertheless, different strains can infect a range of species. Molecular and epidemiological studies have shown that genetically similar isolates can be recovered from sheep, goats and cattle (Collins *et al* 1990; Feizabadi *et al* 1997). Such studies suggest but do not prove that inter-species transmission occurs.

Ovine JD is a chronic disease and infected animals are usually asymptomatic. OJD has a prolonged incubation period, usually 3 to 4 years and animals remain infected for life (Huchzermeyer *et al* 1994). Sheep shed large numbers of bacteria in their faeces, especially in the terminal stages of disease, contaminating pastures. Transmission to susceptible sheep occurs by contact with contaminated pasture (Whittington *et al* 1999).

OJD is endemic in the eastern states of Australia and now subject to a nationally coordinated control program supported by State legislation.

Evidence that *M paratuberculosis* infects ovine and caprine embryos and semen

In the terminal stage of infection *M paratuberculosis* is distributed widely throughout the body (Huchzermeyer *et al* 1994) and embryos could be exposed to infection. Infection can be acquired *in utero* if the disease in the ewe is advanced (Gilmore and Angus 1991). It is unlikely that collected embryos would be infected as collections would only be made from animals which did not show clinical signs.

M paratuberculosis can be isolated from bull semen but there is no specific evidence of *M paratuberculosis* shedding in sheep or goat semen or of transmission by semen.

Probability of *M paratuberculosis* entry, establishment and spread

M paratuberculosis is not typically associated with the genital tract, but intrauterine transmission has been reported. However, the collection of embryos from infected donors showing clinical signs is unlikely. Consequently, the probability of transmission of *M paratuberculosis* to recipients via genetic material is estimated to be negligible for embryos and low for semen.

Probability of adverse consequences arising through *M paratuberculosis* entry, establishment and spread

Sheep and goats are susceptible to *M paratuberculosis*. Losses would result from ill-thrift, cost of eradication and/or control measures. In Australia *M paratuberculosis* has been detected in NSW, Victoria, Kangaroo Island (SA) and Flinders Island (Tasmania) but has not been reported from Queensland, the Northern Territory or Western Australia. The disease is notifiable and subject to compulsory government controls, including quarantine and movement restrictions.

The probability of adverse economic consequences in *M paratuberculosis* free regions in Australia is estimated to be moderate. *M paratuberculosis* was recently cultured from the intestinal tissues of two Tammar wallabies from Kangaroo Island (Primary Industries and Resources South Australia) but the susceptibility of other native Australian animals is unknown and there are no reports of *M paratuberculosis*

in other Australian mammals, birds, reptiles or amphibians. Consequently the probability of adverse environmental consequences is estimated to be low. The overall estimate for probability of adverse consequences is moderate.

Risk estimate for M paratuberculosis

The risk estimate for *M paratuberculosis* without risk management measures is negligible for embryos and low for semen.

4.5 Brucella ovis

Disease caused by *B ovis* affects sheep in Australia and is notifiable. The incubation period is typically prolonged, ranging from 50 to 250 days (van Tonder *et al* 1996).

Male goats have been infected experimentally (Burgess *et al* 1985) but disease in goats is unusual. *B ovis* is not considered a hazard of caprine genetic material in this risk assessment.

Evidence that *B* ovis infects ovine embryos and semen

Ewes are relatively resistant to infection with *B ovis* and infections are transient and generally short-lived. Bacteraemia occurs but rarely results in abortion. When abortion does occur bacteria may be present in uterine tissues and discharges (van Tonder *et al* 1996). Recent experimental and field studies found that the uterus was one of the main sites of infection (Grillo *et al* 1999; Marco *et al* 1994) and that persistent infection did occur. Even so, latent infection and infection of progeny was unusual (Grillo *et al* 1999).

It is not known whether embryos may become infected naturally but *in vitro* exposed embryos transmitted infection to recipient ewes (Riddell *et al* 1990).

B. ovis is shed in semen of infected rams for up to four years (van Tonder *et al* 1996) and inseminated infected semen may cause infection in recipients.

Probability of B ovis entry, establishment and spread

The probability that embryos infected with *B ovis* will infect recipients is estimated to be low. *B ovis* may be present in semen collected from asymptomatic infected donors and transmit infection. In the event that animals become infected through transfer of imported genetic material they can be detected and culled thus preventing establishment and spread. Consequently, the probability that *B ovis* gains entry, establishes and spreads is estimated to be low for embryos and high for semen.

Probability of adverse consequences arising through *B* ovis entry, establishment and spread

B ovis is endemic in many sheep flocks in Australia and causes losses in sheep flocks mainly through poor reproductive performance in rams and their subsequent culling. Losses would also be incurred if eradication and/or control measures were adopted. The introduction of *B ovis* via embryos or semen into regions where *B ovis* free flocks are present, for example stud sheep raising areas, would have a localised impact. However, the impact of introduction into most other areas where the disease is endemic would be minimal. Overall it is estimated that the probability of adverse economic consequences would be negligible. *B ovis* infects mice under laboratory conditions (Jimenez *et al* 1994). There are no reports of *B ovis* infection in native Australian animals, even though the agent is endemic in many regions and the probability of adverse environmental consequences is estimated to be negligible. The overall estimate for probability of adverse consequences is negligible.

Risk estimate for *B* ovis

The risk estimate for *B* ovis in sheep without risk management measures is negligible for embryos and low for semen.

4.6 Brucella melitensis

The usual mode of transmission of *Brucella melitensis* infection is through direct contact with the placenta, foetal fluids or vaginal discharges expelled by infected ewes after abortion or full-term parturition (Garin-Bastuji *et al* 1998). Young animals may recover from infection with *Brucella melitensis* but adults seldom recover, becoming inapparent carriers. Flocks may remain infected for years with a high prevalence of infection even in the absence of obvious disease (Herr 1994).

All caprine breeds appear to be equally susceptible, but susceptibility to *B melitensis* infection varies between breeds of sheep (Herr 1994).

Evidence that B melitensis infects ovine and caprine embryos and semen

B melitensis can localise in uterine tissue of sheep (Grillo *et al* 1997) and cause abortion in goats and sheep. Birth or abortion may be followed by a copious purulent discharge from the genital tract of infected animals for up to 3 months (Herr 1994). Transmission of infection from sheep to lambs occurs. Infection can be acquired *in utero* but the majority of transmission is thought to occur via colostrum or milk (Grillo *et al* 1997).

B melitensis is commonly shed in semen (Garin-Bastuji *et al* 1998) but there is no specific evidence of *B melitensis* transmission via ovine or caprine semen.

Probability of *B melitensis* entry, establishment and spread

There is some evidence to suggest that *B melitensis* may be associated with embryos or semen collected from asymptomatic infected donors. Consequently, the probability that *B melitensis* will gain entry, establish and spread if present in imported genetic material is estimated to be moderate.

Probability of adverse consequences arising through *B melitensis* entry, establishment and spread

Losses arising from the spread of *B melitensis* in sheep and goats result from 'abortion storms', ill thrift, the cost of control and eradication measures. Brucellosis is an important zoonosis. Dogs, cattle and rodents are also susceptible to infection even though less important hosts in epidemiological terms. Consequently, the cost of control and eradication would extend beyond measures applicable to sheep and goat properties and the probability of adverse (socio-)economic consequences due to *B melitensis* spread is estimated to be high. The susceptibility of native Australian animals is unknown. *B melitensis*, in common with other *Brucella* species, has a fairly broad host range and has the potential to cause disease in these species. The probability of adverse consequences is estimated to be high. The overall estimate for probability of adverse consequences is high.

Risk estimate for *B melitensis*

The risk estimate for *B melitensis* without risk management measures is high for embryos and semen.

4.7 Mycoplasma agalactiae and related mycoplasmas (Contagious agalactia)

The principal causal agent of contagious agalactia (CA) in sheep is *Mycoplasma agalactiae*. In goats *M agalactiae*, *M capricolum* subsp. *capricolum* (Mcc), *M putrefasciens*, and *M mycoides* subsp. *mycoides* LC (MmmLC) produce a similar clinical picture and some authors consider these mycoplasmas,

separately or in combination, as causal agents (Bergonier *et al* 1997). In a recent report of CA in Spain *M agalactiae* was isolated from 79% of outbreaks and accounted for 82.7% of all isolates (Gil *et al* 1999). *M capricolum, M putrefaciens* and MmmLC have been isolated from goats in Australia (Cottew and Yeats 1982). For the purposes of this risk analysis only *M agalactiae* is considered further as the cause of CA.

M agalactiae has been detected in the external ear canal of goats in Australia (Cottew and Yeats 1982), but the Australian strains do not appear to produce contagious agalactia in goats or sheep. Avirulent strains have also been reported overseas (Bergonier *et al* 1997).

Goats are more commonly affected than sheep and disease in goats is usually more acute. Long term chronic infections occur in both sheep and goats (Bergonier *et al* 1997). Bacteraemia occurs commonly and *M agalactiae* can be isolated from the blood of infected sheep (Ak *et al* 1995). Clinical signs of CA were evident 20 days after experimental infection of sheep with *M agalactiae* and antibody titres were first detected from 28 to 35 days after infection (Buonavoglia *et al* 1999)

Evidence that *M* agalactiae infects ovine and caprine embryos and semen

Infection involves the genital organs of the female and in goats may cause granular vulvo-vaginitis (Bergonier *et al* 1997; Singh et al 1974). Abortion occurs infrequently. Vertical transmission has been suspected following isolation of *M agalactiae* from swollen joints of neonate kids and lambs (Bergonier *et al* 1997).

M agalactiae was isolated from the semen of experimentally infected sheep (Ak et al 1995).

Probability of *M* agalactiae entry, establishment and spread

Genital tract involvement, abortion, semen infection and suspected vertical transmission have been reported in association with *M agalactiae* infection. The disease spreads quickly once established in a flock (Tola *et al* 1996) and sheep and goats may harbour infection asymptomatically. Consequently the probability that entry establishment and spread of *M agalactiae* would occur via genetic material from infected sheep or goats is estimated to be moderate for embryos and semen.

Probability of adverse consequences arising through *M* agalactiae entry, establishment and spread

CA is considered to be one of the most serious diseases affecting small ruminants (Gil *et al* 1999) characteristically causing localised outbreaks. It is a major obstacle to sheep and goat production in both traditional and intensive forms of stock management (Bergonier *et al* 1997). For example, Tola *et al* (1996) claim that CA has caused major economic loss since introduction into Sardinia in 1980. Spread of the causative agent in sheep and goat flocks in Australia would cause losses due to deaths, loss of production and the cost of control and eradication measures. The probability of adverse economic consequences due to CA is estimated to be moderate. The susceptibility of native Australian animals is unknown. There are no reports of CA in mammals other than ruminants. Consequently the probability of adverse consequences is estimated to be low. The overall estimate for probability of adverse consequences is moderate.

Risk estimate for *M* agalactiae

The risk estimate for *M* agalactiae without risk management measures is moderate for embryos and semen.

4.8 Maedi-visna virus

The ovine lentiviruses, maedi-visna virus (MVV) and South African ovine maedi-visna virus (SA-MVV) can infect sheep causing maedi-visna disease (Banks *et al* 1983). This disease is also called ovine progressive pneumonia, Montana sheep disease, zwoegersiekte, la bouhite, lungers, Marsh's progressive pneumonia and Graaff-Reinet disease. The ovine lentiviruses are closely related to, but genetically and serologically distinct from, caprine arthritis encephalitis virus (CAEV) (Verwoerd and Tustin 1994; Pasick 1998).

MV has been reported from the US (as ovine progressive pneumonia), Canada and most of the countries in the EU. The disease has never been recorded in sheep or goats in Australia and there is no evidence to suggest that the disease is present.

MVV infections are characterised by a long and variable incubation period and life-long viral persistence (Cutlip *et al* 1988). The antibody response confers no resistance to disease and the clinical course of disease is generally progressive (Carey and Dalziel 1993; Verwoerd and Tustin 1994). Natural infection following introduction of MV infected sheep into susceptible flock can occur after 11 months (Houwers *et al* 1987) but clinical signs are rarely seen in sheep less than 2 years old (Constable *et al* 1996).

Differences in breed susceptibility to MVV have been reported (Houwers *et al* 1989). Icelandic breeds appear to be more susceptible than British breeds and Texels and Border Leicester are more susceptible to disease than Columbia sheep (Cutlip *et al* 1986; Joag *et al* 1996). Also, Snowder *et al* (1990) determined significant differences in the seroprevalence of MV between the 6 breed types comprising a flock of 2,976 sheep. Nevertheless, complete breed-associated resistance has not been demonstrated (Houwers 1990). Houwers *et al* (1989) suggest that apparent susceptibility may also depend on the strain of MVV.

MVV infection often co-exists with jaagsiekte and concurrent infection can lead to increased seroprevalence of MV and increased lateral transmission of MVV. Carey and Dalziel (1993) suggest that this may be due to increased alveolar macrophages in the lungs of sheep affected by jaagsiekte providing extra sites for MVV replication.

Evidence that MVV infect embryos and semen

The evidence for transplacental transmission of MVV is equivocal. Preventing colostral transfer and early contact with infected dams has been regarded as an effective means of obtaining MV free progeny (De Boer *et al* 1979; Cutlip *et al* 1988; Sihvonen 1980). Long-term absence of MVV infection was demonstrated in a group of approximately 40 lambs separated from infected ewes immediately after birth and reared in isolation (De Boer *et al* 1979). Similar results were reported by Light *et al* (1979) and Houwers *et al* (1987). Other studies suggest that the potential for transplacental infection cannot be entirely dismissed. Cutlip *et al* (1981) reported prenatal transmission based on the detection of MVV from 1 foetus and 2 newborn lambs out of 70 progeny. Cross *et al* (1975) reported infection in a small proportion of hysterectomy derived lambs from infected dams. More recently, Brodie *et al* (1994) detected MVV DNA in the peripheral blood mononuclear cells (PBMC) of 11% of lambs removed from their infected dams immediately after birth.

Of perhaps greater relevance to an assessment of the potential for infection of ova or embryos with MVV is that viraemia develops shortly after infection and plays a major role in distribution of monocyte associated virus throughout the body (Georgsson 1990). This might provide an opportunity for exposure of embryos to virus. Using PCR techniques, Woodall *et al* (1993) failed to detect MVV in either uterine

washes or washed embryos collected from 10 infected ewes. This suggests that exposure of embryos to MVV during infection does not occur.

Ovine lentivirus was detected in the semen of rams concurrently infected with *Brucella ovis* (de la Concha-Bermejillo *et al* 1996). These authors suggest that inflammatory lesions of the genital tract causing leucocytospermia, as caused by *B ovis*, predispose infected rams to shed ovine lentivirus in their semen. Moreover semen may contain blood or plasma and MVV capsid antigen has been detected in plasma of infected sheep (Brodie *et al* 1994). The target cells for MVV replication are mononuclear cells and transmission of virus occurs via these cells (Joag *et al* 1996). Nevertheless these studies do not provide clear evidence that the MVV components detected are infectious or that infection is transmitted to recipient ewes or offspring via infected semen.

Probability of MVV entry, establishment and spread

Because of the long incubation period, persistent infection, lack of overt clinical signs and genital tract involvement (in males), MVV has the potential to infect genetic material collected from infected donors. Susceptible species are present in this country and it is estimated that the probability of MVV introduction and spread in this country is low for embryos and moderate for semen.

Probability of adverse consequences arising through MVV entry, establishment and spread

MVV infection causes ill-thrift, chronic respiratory disease and indurative mastitis in adult sheep and reduced growth rates in lambs. In flocks with high prevalence the disease is economically significant and measures have been adopted overseas to eradicate MVV. Spread of MVV in sheep in this country would cause losses due to deaths, 'ill thrift' and the cost of control and eradication measures. Snowder *et al* (1990) studied reproductive performance, ewe weight and wool production in 6 breed types in the USA and concluded that subclinical infection did not appear to have an adverse economic effect. Even so, the probability of adverse economic consequences due to MVV is estimated to be moderate. The susceptibility of native Australian animals is unknown and there are no reports of MVV in animals other than small ruminants. Consequently the probability of adverse environmental consequences is estimated to be low. The overall estimate for probability of adverse consequences is moderate.

Risk estimate for MVV

The risk estimate for MVV without risk management measures is low for embryos and moderate for semen.

4.9 Caprine arthritis-encephalitis virus

Caprine arthritis encephalitis (CAE) is also called chronic arthritis-synovitis, big-knee, viral leukoencephalomyelitis, progressive interstitial pneumonia and caprine retrovirus disease. Goats infected with CAE virus are usually asymptomatic. The disease is usually expressed in adults as a chronic arthritis, and occasionally as progressive interstitial pneumonia or chronic mastitis. The disease is enzootic in goats in Australia (Greenwood *et al* 1995) but voluntary control programs are in place in some parts of the country.

CAEV and MVV are generally regarded as closely related but separate viruses. Strong evidence that Australasian strains of CAEV do not transmit from goats to sheep is provided by separate studies in Australia and New Zealand. Smith *et al* (1985) failed to demonstrate the development of any significant pathological lesions in Merino lambs exposed to a Western Australian strain of CAEV by inoculation and through close contact with infected goats. 1394 sheep in New Zealand exposed to goat flocks with a moderate to high seroprevalence of CAE tested negative for CAEV infection (McKenzie 1991). However

some other studies suggest that overseas strains of CAEV may infect sheep and some authors refer broadly to small ruminant lentiviruses, regarding MV viruses and CAE virus as separate viral 'quasispecies' (Pasick 1998). Experimental infections of sheep with CAEV have been reported (Banks *et al* 1983; Castro et al 1999) and disease signs typical of CAE have been observed in sheep (Oliver 1981). Based on phylogenetic studies of nucleic acid sequences of overseas isolates of CAEV and MVV, Zanoni (1998) suggests that there are clear indications for cross-species transmission and advises that eradication programs should be aware of this risk. Consequently, the potential for strains of CAEV not present in this country to infect sheep cannot be entirely dismissed.

CAE is a progressively debilitating disease with life-long viral persistence (Verwoerd and Tustin 1994). The arthritic form of CAE predominates and is usually expressed in adult goats when they are 2 to 9 years old (Narayan and Cork 1990).

Evidence that CAEV infects embryos and semen

Colostrum and milk from infected goats usually contain infected cells (Narayan and Cork 1990) and eradication efforts are usually based on colostrum deprivation (Nord *et al* 1998). However, there is no evidence of infection of the female reproductive tract or transplacental transmission.

CAEV has been detected by PCR in seminal fluid and non-spermatic cells in semen from experimentally infected bucks (Travassos *et al* 1998).

Probability of CAEV entry, establishment and spread

CAEV has been detected in caprine semen. A monocyte associated viraemia is typical of CAE and could expose ova or embryos to virus during infection. However, this appears unlikely as in one experiment, CAEV was not isolated from uterine flushings from seropositive does and transferred embryos did not seroconvert recipients or progeny (Wolfe et al 1987). Susceptible species are present in this country and could be exposed to introduced exotic strains of the virus. The probability of CAEV introduction and spread in this country is estimated to be low for embryos and moderate for semen.

Probability of adverse consequences arising through CAEV entry, establishment and spread

CAEV infection causes neurological problems in young goats and ill-thrift and chronic lameness in adult goats. The disease is considered to be economically significant such that control measures have been adopted overseas and in Australia. Introduction and spread of strains of CAEV which cause disease in sheep could cause losses due to deaths, 'ill thrift' and the cost of control and eradication measures. The probability of adverse economic consequences due to the introduction of overseas strains of CAEV is estimated to be moderate. Except for goats and sheep, there is no other known host for CAEV (Narayan and Cork 1990). Consequently the probability of adverse environmental consequences is estimated to be low. The overall estimate for probability of adverse consequences is moderate.

Risk estimate for CAEV

The risk estimate for CAEV without risk management measures is low for embryos and moderate for semen.

4.10 Mycoplasma capricolum subsp. capripneumoniae (contagious caprine pleuropneumonia)

The causative agent of contagious caprine pleuropneumonia (CCPP), *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) was formerly known as the F38 biotype (Leach *et al* 1993). The diagnosis of CCPP is complicated as disease resembling CCPP in goats may be caused by *M mycoides* subsp. *capri*

(Mmc) and caprine variants of *M mycoides* subsp. *mycoides* (MmmLC). Mccp, Mmm and Mmc cross react in serological tests and are grouped together with bovine serogroup 7 and *M capricolum* as the "*M mycoides* cluster' (Nicolet 1994; Taylor *et al* 1992). MmmLC, *M capricolum* and Mmc have been recorded in Australia (Beveridge 1983; Taylor *et al* 1992).

CCPP is transmitted by the respiratory route and spreads rapidly within a herd by close direct contact (Geering *et al* 1995; MacOwan and Minette 1977). The incubation period for the disease, ranges from 3 to 9 days after experimental infection and 12 to 35 days after natural contact (MacOwan and Minette 1977). A much longer incubation period, from 24 to 50 days, was reported in animals previously exposed to high passage culture of Mccp (MacOwan and Minette 1978). Chronic infections can occur and apparently healthy carriers can excrete mycoplasmas (Geering *et al* 1995; Nicolet 1994). Another experimental study showed that goats recovered from acute CCPP may have lesions for a long time thereafter but provided no evidence of a carrier state among long-term survivors (Wesonga *et al* 1998). Mccp has been isolated from asymptomatic sheep held in affected goats herds but sheep are considered to be resistant to disease (Harbi *et al* 1983; Mare 1994). However, Bolske *et al* (1995) reported the isolation of Mccp from sheep with typical signs of CCPP.

Evidence that Mccp infects caprine embryos and semen

Mccp localises in the respiratory tract and does not cause septicaemia or pathology in the genital tract. There is no evidence to suggest that embryos are exposed to Mccp in naturally infected animals.

Untyped mycoplasmas have been recovered from semen and preputial swabs from bucks (Kapoor *et al* 1983). There is no other specific information which can be used to estimate the potential for transmission of Mccp via caprine semen.

Probability of Mccp entry, establishment and spread

Mccp is typically a respiratory disease so the probability of infection of embryos is estimated to be low. Based on very limited information it is estimated that the likelihood Mccp is present in semen from infected animals is low. CCPP spreads rapidly and efficiently among susceptible animals by direct contact. Should infected genetic material cause infection in recipients or progeny the likelihood that the disease would spread is high. In summary, the probability that Mccp will gain entry, establish and spread is estimated to be low for semen and embryos.

Probability of adverse consequences arising through Mccp entry, establishment and spread

CCPP is a disease of major economic importance, posing a significant restraint to goat production due to high mortalities and rapid spread. Large scale treatment has proven ineffective in eradicating the disease in endemic areas (Bolske *et al* 1995). Feral goats (*Capra hircus*) occur in all states and territories of Australia except the Northern Territory. The main populations are in western New South Wales, the semi-arid pastoral areas of Western Australia and southern Queensland. Spread of Mccp in goats in this country would cause losses due to deaths, 'ill thrift', the cost of control and eradication measures. The economic value of the goat industry is small in comparison with other livestock industries. Therefore, the probability of adverse economic consequences due to Mccp on a national level is estimated to be low. The susceptibility of native Australian animals is unknown but there are no reports of Mccp in mammals other than goats. Consequently the probability of adverse environmental consequences is estimated to be low. The overall estimate for probability of adverse consequences is low.

Risk estimate for Mccp

The risk estimate for Mccp without risk management measures is low for embryos and semen.

4.11 Chlamydia psittaci (enzootic abortion of ewes)

Chlamydia psittaci isolates from ruminants have been split into 2 groups based on their virulence in mice (Salinas *et al* 1995). Strains virulent for mice form a well defined group and have been designated as serotype 1. Ovine abortion strains causing enzootic abortion of ewes (EAE) are placed within this serotype (Kaltenboeck *et al* 1997; Boumedine and Rodolakis 1998). The taxonomy of the family *Chlamydiaceae* has been recently reviewed and Everett *et al* (1999) propose that ovine abortion strains are included in the newly constructed species *Chlamydophila abortus*. Goats as well as sheep can be affected by ovine abortion strains of *C psittaci* (Salinas *et al* 1995; Liao *et al* 1997; Donn *et al* 1997).

No evidence of chlamydial infection of aborting ewes or dead lambs was found in a large scale study of perinatal mortality and ovine abortion in NSW (Plant *et al* 1972). The recovery of isolates indistinguishable from abortifacient *C psittaci* in sheep flocks free of abortion in the UK and in Greece suggests the existence of avirulent forms of ovine *C psittaci* (Jones 1997). Naturally occurring sporadic abortion episodes due to chlamydial infections have been recorded in sheep and goats in Australia (Brown *et al* 1988; Seaman 1985). Even so, the strains of *C psittaci* that cause typical enzootic abortion have not been reported in Australia.

EAE appears to have a minimum incubation period of 56 days (Williams *et al* 1998). There is no evidence of breed predisposition to EAE.

Evidence that C psittaci infects embryos and semen

C psittaci is a naturally occurring reproductive infection in sheep that persists following primary infection. *C psittaci* antigen or DNA was detected in the vagina, uterus and oviduct of experimentally infected sheep (Papp and Shewen 1996). *C psittaci* infection involves the female genital tract and neonatally-infected ewe lambs may develop placental infection during their first pregnancy and some may abort (Aitkin 1991). *C psittaci* has also been isolated from abortion outbreaks in goats (Liao *et al* 1997; Vretou *et al* 1996), cattle (Griffiths *et al* 1995) and humans. The disease in sheep is characterised by a long-term persistent phase (Entrican *et al* 1999).

Chronic infection of the male reproductive tract and seminal excretion can occur in ruminants (Storz *et al* 1976). *C psittaci* has been isolated from semen of young rams born to experimentally infected ewes (Appleyard *et al* 1985). Venereal transmission under experimental conditions has been reported. In one experimental study, nine out of ten ewes inseminated with semen to which *C psittaci* was added seroconverted and the organism was recovered from 3 of these. Fourteen ewes served by four rams intravenously infected with *C psittaci* seroconverted but the agent was not recovered at any stage from served ewes. These authors concluded that venereal transmission of *C psittaci* is biologically feasible in sheep (Appleyard *et al* 1985) but is not a major route of spread.

Probability of C psittaci entry, establishment and spread

C psittaci infection involves the female genital tract and vertical transmission of infection from ewes to lambs can occur. EAE spreads easily from infected aborting ewes to unaffected ewes under intensive lambing conditions. Transmission by ET has not been reported. The probability of entry, establishment and spread of *C psittaci* via infected embryos is rated as moderate.

There is no evidence to suggest that rams or bucks play a significant role in the transmission of EAE (Appleyard *et al* 1985) or that transmission via AI has occurred. However seminal excretion and venereal transmission in ruminants have been reported and the probability of entry, establishment and spread of *C psittaci* via infected semen is rated as moderate.

Probability of adverse consequences arising through C psittaci entry, establishment and spread

EAE is a major cause of economic loss to sheep farmers in the UK and several other European countries, especially when intensive sheep management is practiced (Aitken 1991). Other ruminant species are susceptible to chlamydial abortion (Aitkin 1991) but the taxonomic relationship of causative chlamydia in other ruminants to that causing EAE is uncertain. All breeds of sheep would be expected to be susceptible to *C psittaci* but expression of disease is dependent on the intensity of management at lambing. In Australia most lambing takes place in paddocks and pregnant ewes are not confined together. Therefore the economic effect of EAE spread in Australia is estimated to be low. Under natural conditions chlamydial species are considered to have a relatively limited host range and relatively limited disease range (Everett and Anderson 1999). Nevertheless, the ovine abortion strain of *C psittaci* has apparently caused abortion in humans and cattle. *C psittaci* strains infect Australian birds and cause disease in koalas (Girjes *et al* 1993). However, the relationship between these strains and that causing EAE is unclear. It is concluded that the susceptibility of native Australian animals to EAE is unknown and the probability of adverse environmental consequences estimated to be low. The overall estimate for probability of adverse consequences is low.

Risk estimate for *C psittaci*

The risk estimate for ovine abortion strains of *C psittaci* without risk management measures is low for embryos and semen.

4.12 Jaagsiekte virus

A type D retrovirus, called jaagsiekte sheep retrovirus (JV) is the aetiological agent of a contagious lung tumour of sheep. The disease is known as jaagsiekte, ovine pulmonary adenomatosis, ovine pulmonary carcinoma, sheep pulmonary adenocarcinoma and sheep pulmonary adenomatosis. There is still some controversy about the pathological classification of the lung tumour (Verwoerd 1996) and the original descriptive name jaagsiekte is often used. The disease has been experimentally reproduced in lambs by infection with a jaagsiekte virus (JV) cloned construct (Palmarini *et al* 1999). Endogenous and exogenous forms of the virus exist with differing nucleotide sequences. Three distinct groups have been identified;

- i) exogenous JV sequence from the UK,
- ii) exogenous JV sequences from Southern Europe and
- iii) the exogenous South African strain plus all the endogenous sequences analysed and collected from sheep in Australia, Italy, UK and South Africa (Rosati *et al* 2000). The endogenous retroviral sequences are present within the ovine genome and distinct from JV (Bai *et al* 1999).

The prevalence of jaagsiekte in sheep can be high in some countries, for example, UK (Scotland) (Hunter and Munro 1983) and the Netherlands (Houwers and Terpesta 1984). Even though the disease has been experimentally transmitted from sheep to goats the disease is uncommon in goats and the risk of transmission is regarded as low (Sharp *et al* 1986).

The influence of genetics on resistance to jaagsiekte is poorly understood. Some breeds are apparently more susceptible to disease than others, eg. English breeds, Texel, Merinos and Awassi (Parker *et al* 1998; Hod *et al* 1972; Hunter and Munro 1983). The presence of endogenous viruses in sheep genomes may influence the susceptibility of sheep to the disease (Hecht *et al* 1996).

The incubation period following experimental infection ranges from 3 weeks in lambs to several years in older animals. Several studies determined the peak incidence of jaagsiekte in sheep to be 3 to 4 years

(Hunter and Munro 1983; Parker *et al* 1998). The average duration of clinical signs is 2 months, with a range of a few days to 6 months (Moulton 1990).

Evidence that JV infects ovine and caprine embryos and semen

JV has not been detected in embryos or uterine fluids. However, during the later stages of disease retroviral transcripts are widely distributed in the tissues of affected animals, including peripheral blood mononuclear cells (PBMC). Based on these findings, Parker *et al* (1998) suggested that exposure of embryos, ova and semen to virus was possible.

The distribution of retroviral transcripts could include genital tract tissues and semen could be infected. The only study conducted to investigate this found that the transfer of infection from one diseased ram to progeny and recipients did not occur (Parker *et al* 1998). JSV has been detected in lymphoid tissues, including PBMC and dissemination of virus by these cells is thought to precede tumour formation (Holland *et al* 1999). Any concurrent disease process in a JV infected ram which increases the mononuclear content of semen may lead to the production of JV infected semen.

Probability of JV entry, establishment and spread

Because of the long period before overt clinical signs are detectable, JV may be present in genetic material collected from asymptomatic infected donors. It is estimated that the probability of JV introduction and spread via embryos is low and via semen moderate.

Probability of adverse consequences arising through JV entry, establishment and spread

Jaagsiekte causes significant problems in sheep in countries in which it is enzootic. It is naturally highly transmissible among sheep causing respiratory disease followed by fatality in over 95% of cases. Because of the long preclinical incubation period and contagious nature of jaagsiekte as well as the short life span of most sheep under modern management systems, JV may enter and disseminate widely in a flock before the disease is recognised. Economic losses can arise from mortalities, the cost of control and eradication measures. The probability of adverse economic consequences due to jaagsiekte is estimated to be high. The susceptibility of native Australian animals is unknown and there are no reports of jaagsiekte in mammals other than small ruminants. Consequently the probability of adverse environmental consequences is estimated to be low. The overall estimate for probability of adverse consequences is moderate.

Risk estimate for JV

The risk estimate for JV without risk management measures is low for embryos and moderate for semen.

4.13 Louping-ill and related viruses

Viruses of the tick-borne encephalitis (TBE) complex within the flavivirus genus occur across Europe to North-East Asia and can cause disease in humans (Kaiser *et al* 1997) and significant losses in small ruminant production (Davies 1997). Closely related viruses within this group cause louping ill (LI), Russian spring-summer encephalitis (RSSE) and central European encephalitis (CEE) in some European countries (Monath and Heinz 1996). Hubalek *et al* (1995) propose that these viruses represent one virus species named Eurasian TBE virus (*Flavivirus ixodes*) and group LI and CEE together as a Western subtype. Relationships between LI and other members of the TBE complex, including Turkish sheep encephalitis (TSE) and Spanish sheep encephalitis (SSE), are unclear. For the purposes of this risk assessment the louping ill virus (LIV) and louping-ill like viruses which affect sheep and goats (TSEV and SSEV) are regarded as forming a separate sub-group or complex of genetically distinct but closely related sheep encephalitis viruses. This grouping is largely based on clinical signs, serology and host range (Marin *et al* 1995; Hubalek *et al* 1995) and referred to here as louping ill and related viruses (LIRV). These viruses are

reported from the UK, Spain, Bulgaria, Turkey, Norway and Greece (Marin *et al* 1995; Gao *et al* 1993, 1997; Papadopoulos *et al* 1971).

LI is predominantly a disease of sheep but can affect goats. The incubation period following experimental infection, taken as the onset of viraemia, varies from one to eleven days in sheep (Swanepoel 1994; Reid and Doherty 1971) and from one to six days in goats (Reid *et al* 1984). Following the primary viraemic phase, LIV replicates in both neural and extraneural tissue but is strongly neurotropic and can sequester in nervous tissue (Monath and Heinz 1996).

TBE viruses are naturally transmitted by ticks within genera *Ixodes*, *Dermacentor* and *Haemaphysalis*. Geering *et al* (1995) suggest that the viruses are unlikely to become established in this country unless suitable vectors are first introduced. However, Swanepoel (1994) suggests that louping ill virus, in common with other tick-borne viruses, may be capable of transmission by a wide range of ticks.

Evidence that LIRV infects ovine and caprine embryos and semen

A TBE complex arbovirus was isolated from a flock of goats with abortions (Papadopoulos *et al* 1971). However, there is no evidence that LIV can infect ruminant embryos or embryonic fluids.

No information is available on the presence of LIV in ovine or caprine semen.

Probability of LIRV entry, establishment and spread

LIV causes a viraemia and has the potential to infect genetic material but the likelihood that infected genetic material may cause disease in recipients or offspring is unknown. The likelihood that disease may spread from index cases depends on the capacity of biological vectors in this country to transmit LIRV. There is very little evidence of the movement of strains of LIV from different areas of UK despite substantial trade of livestock. Other viruses within the TBE complex tend to remain geographically restricted despite movement of humans and animals. This suggests that the geographical distribution of LIRVs is determined by the distribution of ticks, that the viruses are vector specific and that spread of viruses from specific localities is unusual. Overall, the probability that LIRV establish and spread via infected genetic material is estimated to be negligible.

Probability of adverse consequences arising through LIV entry, establishment and spread

LIV is locally important in some areas of Europe but is not generally regarded as a significant disease affecting sheep. However, LIV and probably the other LIRVs pose a risk of human infection. It does not transmit without intervention of a competent vector and the capacity of arthropods in Australia to fill this role is uncertain. Consequently the probability of adverse (socio-) economic consequences due to LIV is estimated to be low. The susceptibility of native Australian animals is unknown and LIV has a wide host range (Davies 1997). However the uncertainty of transmission is also relevant here. Consequently the probability of adverse is estimated to be low. The overall estimate for probability of adverse consequences is low.

Risk estimate for LIRV

The risk estimate for LIRV without risk management measures is negligible for semen and embryos.

4.14 Leptospira spp

Leptospirosis is a zoonotic disease with a worldwide distribution caused by pathogenic species of *Leptospira*.

Leptospirosis has been reported in sheep and/or goats in many European countries and the US. In Portugal 3.3% of sheep tested gave positive results. The predominant serogroups involved were Canicola, Pomona, Cynopteri, Sejroe and Icterohaemorrhagiae. From the 1,631 goats examined serologically, 5.0% gave positive results, mainly to serogroups Grippotyphosa, Canicola, Pomona, Icterohaemorrhagiae and Pyrogenes (Rocha 1998). Infection of sheep has been reported in Italy (*L hardjobovis* and *L interrogans* sv hardjo) (Farina *et al* 1996; Cerri *et al* 1996), the Netherlands (*L interrogans* sv hardjo and *L hardjo bovis*) (Gerritsen *et al* 1995), UK (Sejroe and hebdomadis serogroups, sv bratislava and autumnalis) (Little *et al* 1987; Hathaway *et al* 1983), Spain (sv pomona, sejroe, icterohaemorrhagiae and grippotyphosa) (Leon-Vizcaino *et al* 1987) and France (Gaumont 1966).

In one study in the US, leptospiral antibody titres were present in 27 of 326 ovine foetal sera tested. However, leptospires were not detected in any of the 326 ovine foetuses tested by FAT (Kirkbride and Johnson 1989). *L interrogans* sv hardjo and pomona have been reported in sheep in the US (Schmitz et al 1981; Davidson and Hirsh 1980).

Sporadic cases of leptospirosis in sheep in Australia are caused by *L borgpetersenii* sv hardjo and members of the hebdomadis serogroup (McCaughan *et al* 1980). Serosurveys have indicated that serovars tarassovi, pomona, australis, autumnalis, copenhageni, grippotyphosa and hardjoprajitno (*L interrogans* sv hardjo) may be present in sheep (Gordon 1980).

Infection with pathogenic leptospires may cause a range of clinical signs, from asymptomatic to acute and fatal. Chronic infections can occur and affected animals may remain carriers for long periods with bacteria localising in the kidneys or reproductive tract.

Evidence that leptospires infect ovine and caprine embryos and semen

Leptospires could not be recovered from the reproductive tracts of ewes and rams experimentally infected with *L interrogans hardjo* (Farina *et al* 1996). In addition, neither venereal transmission nor recovery from semen has been demonstrated in sheep and goats (Anon 1981).

A very small percentage of abortions in sheep and goats were attributed to infection with leptospires in Spain and the UK (Leon-Vizcaino *et al* 1987; Ellis *et al* 1983). One more recent, large study in the US failed to detect leptospires in 326 aborted ovine foetuses in spite of serological evidence of infection (Kirkbride and Johnson 1989). There is no published information on leptospire transmission in ruminants by ET.

Probability of leptospire entry, establishment and spread

Leptospira can be detected in bovine semen (Heinemann *et al* 1999). However, transmission via AI in ruminants has not been recorded and Philpott (1993) cites evidence that freeze-thawing destroys leptospires in semen. Palit *et al* (1986) found that glycerol which is used as a cryoprotectant in ruminant semen was toxic to the majority of *Leptospira interrogans* serovars tested. This indicates that the probability leptospires will be present in semen and survive normal processing is negligible. Similarly, normal processing of embryos should preclude contamination with leptospires. Overall, the probability that exotic pathogenic serovars of *Leptospira* establish and spread via infected genetic material is estimated to be negligible.

Probability of adverse consequences arising through leptospire entry, establishment and spread

Of the serovars reported in sheep and/or goats overseas, several have not been reported in Australia. Even though leptospirosis is endemic in Australia, introduction of these exotic strains may pose a public health

risk and could cause loss of production in naive animals. However, the pathogenicity of the exotic serovars under Australian conditions is unknown and the significance of leptospirosis in this country mainly derives from the zoonotic potential. Consequently, the probability of adverse consequences is estimated to be low.

A wide range of leptospire serovars has been recovered from native Australian animals without symptoms (Glazebrook *et al* 1978) indicating that the probability of adverse environmental consequences is negligible. The overall estimate for probability of adverse consequences is low.

Risk estimate for leptospires

The risk estimate for pathogenic serovars of leptospires not present in Australia without risk management measures is negligible for semen and embryos and risk management measures are not required.

5. RISK MANAGEMENT OPTIONS

Risk management options considered in this section are additional to the recommendations of IETS and OIE (appendices 4.2.2.2. and 4.2.3.3.) for the collection and processing of semen and embryos. The OIE appendices refer to artificial insemination (AI) centres, embryo collection teams and processing laboratories. Measures recommended for collection include donor flock/herd freedom, animal testing and embryo washing as described in the IETS Manual (Stringfellow 1998).

5.1 Disease freedom of animals in country, zone or flock

Reliance on country freedom to ensure minimal risk of disease transmission via implantation or insemination of genetic material assumes that adequate disease surveillance and veterinary controls are in place in that country. The length of time that the flock of origin has been in existence, the frequency and source of recruitment to the flock and the period of residence of the donor in the flock of origin are also relevant.

The geographical distribution of disease agents for the purposes of this risk analysis, is obtained from the most recent OIE annual reports and other scientific reports.

The OIE *Code* definition of flock/herd freedom for each disease agent is used when available. Flock/herd freedom is relied on as a risk reduction option when individual animal testing is not useful. This arises when there are no reliable ante-mortem methods for detecting infected animals and where available serological tests are of low sensitivity.

5.2 Washing embryos

Special sanitary measures required for *in vivo* production of embryos include the washing of embryos. The washing procedure, as described in the IETS Manual (Stringfellow 1998), usually removes all traces of pathogens associated with embryos or picked up by the embryo during uterine flushes. The addition of trypsin assists in the removal of pathogens, such as certain enveloped viruses, from the surface of the ZP during the washing process. Appropriate antibiotics can be used in the medium to reduce populations of some bacteria and mycoplasmas. Certain viruses and bacteria have been found to adhere so firmly to the ZP that even standard washing procedures may fail to remove them. This appears to be true for *Brucella ovis* in sheep and MmmLC in goats (Guerin *et al* 1997). Once hatched from the ZP the embryo could become infected by these pathogens.

The Research Subcommittee of the IETS import/export committee has categorised some of the pathogens of sheep and goats in terms of the risk of transmission via embryo transfer. An explanation of each of the four categories and sheep and goat diseases grouped within each of these categories is provided at

appendix 2. This information was not provided in the previous section as risk assessments were made assuming that no risk management measures were imposed, ie. that the embryos were not necessarily handled between collection and transfer according to IETS recommendations.

5.3 Testing and examination

Donors and/or the donor's flock/herd of origin may be clinically examined for signs of disease or tested using serological or pathogen detection methods. Semen and embryos or embryo collection fluids can also be tested for the presence of pathogens.

Determination of the infection status of individual donor animals pre-collection, at the time of collection or post-collection may be carried out. Sampling times are based, wherever possible, on a knowledge of incubation periods, the duration of infection and host antibody responses following infection.

Generally, standard culture or virus isolation methods as well as PCR techniques can be applied to semen samples and embryo collection and washing fluids samples.

5.4 Vaccination

Three factors are considered before accepting the use of vaccines in donor animals as risk management options, viz, whether the vaccine:

- protects animals from infection or disease
- is live or killed
- is manufactured according to the methodology in the OIE Manual.

5.5 Treatment

Appropriate antibiotics can be used to treat infected animals prior to collection or added to the medium in which the genetic material is suspended to reduce populations of some bacteria and mycoplasmas. Precollection treatment is only considered as a useful quarantine risk management option if the treatment can completely eliminate the infection. This option is not included as a risk management measure for the pathogens considered in this IRA as the efficacy of available treatments cannot be guaranteed.

5.6 Pre-export quarantine (PEQ)

Holding animals in a defined flock or herd of origin according to the requirements in Appendix 4.2.3.3. during the pre-collection period or in an officially controlled premises, eg. an AI centre, is a form of pre-export quarantine. The major value of this measure is to provide an opportunity for expression of disease in susceptible animals, the prevention of exposure to new infections and the collection of samples for testing.

5.7 Post-arrival quarantine (PAQ)

Recipients of imported genetic material and their progeny may be subjected to a period of quarantine after arrival of the genetic material in Australia. Testing and clinical assessment of recipients and/or progeny can be carried out during this period. This option has not been included for any of the pathogens considered in this IRA. Other risk reduction measures are considered to be more efficacious and practical.

6 RISK MANAGEMENT FOR EACH PATHOGEN

6.1 Foot and mouth disease virus

Disease freedom of animals in country, zone or flock

All countries considered in this IRA are recognised by the OIE as FMD free countries without vaccination. The last reported occurrence of FMD in Greece was in 1996 prior to eradication by stamping out with no vaccination.

Washing embryos

Thibier and Guerin (1999) report on recent unpublished research which indicates that washing embryos may reduce the risk of FMDV transfer with infected embryos. In one study embryos obtained from 31 naturally infected ewes mated to infected rams were washed and 50 were assayed and found to be negative to FMDV. The remaining 25 embryos were cryopreserved and transferred to recipients. All recipients remained uninfected as did the 4 progeny. Another study found that 35% of sheep embryos tested were found positive after *in vitro* exposure to FMDV, but there is no information on whether these embryos were washed. These same workers reported that 185 embryos collected from 19 FMD infected sheep and washed 10 times were negative on FMDV testing.

There is clear evidence that washing removes FMDV from bovine embryos (Mebus and Singh 1988). The structure of the ZP differs in sheep and goats embryos and the value of embryo washing in small ruminants is less certain. This uncertainty is reflected in the IETS ranking of FMD as a Category 1 disease for cattle but Category 3 for sheep and goats.

Testing and examination

Testing is not included for the risk management of FMD.

Vaccination

Vaccination against FMD is not used as frequently in sheep and goats as it is in cattle and the reaction of sheep and goats to vaccination is unclear. Vaccinated cattle may become infected with the virus, despite having protection against the disease, and may spread the virus (Hutber *et al* 1999). Antibodies to FMDV can be detected in vaccinated animals for up to three years after vaccination. These disadvantages would be expected to apply to vaccination in sheep and goats and reduce the value of this risk management measure.

Pre-export quarantine

Pre-export quarantine is not included as a risk management option for FMD.

Measures for embryos

During the 12 months immediately prior to collection the donors lived only in countries or zones recognised by the OIE as being free from FMD. The embryos were collected, washed, processed and stored in accordance with OIE Animal Health *Code* (Appendix 4.2.3.3.).

Measures for semen

During the 12 months immediately prior to collection the donors lived only in a countries or zones recognised by the OIE as being free from FMD.

6.2 Bluetongue virus

Disease freedom of animals in country, zone or flock

Country or territory freedom is defined in Article 2.1.9.2. of the *Code*. Of the countries considered in this risk analysis, bluetongue (BT) has been reported recently in Greece, Canada and the USA. Australia recognises seasonal zone freedom for the bluetongue low incidence States in the USA and Canada.

Washing embryos

The transfer of BTV to recipient sheep via *in vitro* exposed embryos and also embryos from infected ewes has been described (Gilbert *et al* 1987). Other studies have demonstrated that washing embryos according to IETS recommendations prevents the transmission of BTV via the transfer of embryos from viraemic sheep donors. Hare *et al* (1988) failed to transfer infection to seronegative ewes with 39 embryos from infected donors bred by infected rams. Singh *et al* (1997) demonstrated that *in vitro* and naturally exposed ovine embryos did not remain infected after washing nor transmit disease to seronegative ewes or their lambs.

Transfer of washed embryos from a group of does in which 47% tested positive to the virus by serology failed to transmit BTV to susceptible goats and their offspring (Chemineau *et al* 1986).

Published research indicates that the washing of embryos prevents ET transmission of BTV in sheep and goats, however until further research has confirmed this, additional risk management measures are considered warranted. IETS regards BT in sheep as Category 2, and Category 4 in goats.

Testing and examination

BTV infections may be diagnosed by direct virus isolation or by using serological techniques.

BT virus can be recovered from blood samples taken from febrile animals by culture on embryonated chick eggs. PCR techniques can be used to detect virus in blood samples and can be used to determine the serotype of orbiviruses if this information is required (Wade-Evans 1990).

Antibodies are usually first detected from 1 to 2 weeks after infection (*Manual*). The AGID and indirect ELISA test lack specificity in that both tests detect antibodies to other orbiviruses, particularly those in the EHD serogroup. The use of monoclonal anti-BTV antibodies in a competitive ELISA (cELISA) avoids these non-specific reactions (Jeggo *et al* 1992) and this test is now the recommended prescribed test for international trade (*Code*; Reddington *et al* 1991).

Roberts et al (1993) noted that a serological response may be absent in some infected animals.

Vaccination

Attenuated live vaccines are currently used in several countries and provide some protection from disease in sheep. It is unclear whether or not the attenuated viruses in vaccines are transferred via semen or embryos.

Pre-export quarantine

Sheep or goats in quarantine should be protected from *Culicoides* sp. for at least 60 days before collection of embryos or semen. This can be achieved by holding animals in insect-proof premises in a BTV free or seasonally free country/zone. The OIE *Code* recommendations for artificial insemination centres for small ruminants (Article 4.2.2.2.) include insect proofing or spatial or temporal isolation from vector activity.

Measures for embryos

Donors

met the requirements laid down in *Code* Article 2.1.9.11., 2.1.9.12. or Article 2.1.9.13. as appropriate which require certification of embryo collection and processing and that the donor female was either sourced from a BTV free country/zone/quarantine premise or tested negative and

the embryos were collected, washed, processed and stored in accordance with OIE Animal Health *Code* (Appendix 4.2.3.3.).

Measures for semen

Donors

met the requirements laid down in *Code* Article 2.1.9.7., 2.1.9.8 or 2.1.9.9. as appropriate which require certification of semen collection and processing and that the donor male was either sourced from a BTV free country/zone/quarantine premise or tested negative.

6.3 Capripoxvirus

Disease freedom of animals in country, zone or flock

The Code defines country freedom from capripox (sheep and goat pox) when the disease has not been reported for at least the past 3 years. This reduces to 6 months after the slaughter of the last affected animal for countries in which a stamping-out policy is practised with or without vaccination against capripox. Greece is the only country of those considered in this IRA in which recent outbreaks of capripox have occurred. In 1997, 58 outbreaks of sheep pox occurred. Two outbreaks occurred in July 1998. Stamping out of affected and in-contact sheep and goats was used in all cases as a control measure.

Washing embryos

There is no information on the effect of washing on the transfer of capripoxvirus via embryos. Capripoxvirus has not been classified by IETS.

Testing and examination

The virus neutralisation test (VNT) is described in the OIE Manual for the detection of animals infected with capripoxvirus. However the immune reaction is predominantly cell mediated and serological tests may not detect infected animals with low levels of antibody. Western blotting of serum is apparently more sensitive and specific than the VNT (Chand *et al* 1994). However this test is of limited practical value as it is expensive and difficult to perform. An immunocapture ELISA and PCR techniques have been used to detect capripoxvirus in tissues, but application of these tests to semen or embryos has not been reported (Ireland and Binepal 1998; Mangana-Vougiouka *et al* 1999; Rao *et al* 1997).

Vaccination

Live attenuated vaccines using a single strain of capripoxvirus protect both sheep and goats from infection with all field strains of virus. Inactivated vaccines are less effective than live virus in stimulating the cell-mediated immune response which is the predominant protective response to poxvirus infection. There are problems associated with the use of live vaccines, eg. genetic material from vaccinates may harbour live virus. A sub-unit vaccine has been developed which avoids these difficulties but unfortunately it does not completely protect animals from infection (Carn *et al* 1994).

Pre-export quarantine

The incubation period of capripox is 8 to 12 days (Munz and Dumbell 1994) but given as 21 days in the *Code*. Infected animals may be detected if they express disease while held in PEQ during the pre-collection period. Therefore PEQ may be of some value as a risk reduction measure for capripox.

Measures for embryos

During the 6 months immediately prior to collection donors lived only in countries free from sheep and goat pox in accordance with *Code* Article 2.1.10.2.

and

the embryos were collected, washed, processed and stored in accordance with OIE Animal Health *Code* (Appendix 4.2.3.3.).

Measures for semen

During the 6 months immediately prior to collection donors lived only in countries free from sheep and goat pox in accordance with *Code* Article 2.1.10.2.

6.4 Mycobacteria paratuberculosis

Disease freedom of animals in country, zone or flock

There are no *Code* recommendations for trade in semen or embryos in respect of paratuberculosis. The *Code* provides recommendations for trade in domestic ruminants, including requirements on which "flock freedom" may be based (Article 3.1.6.1.) but does not include definitions of country freedom.

Washing embryos

The risk of transmission of *M paratuberculosis* has not been determined by the Research Subcommittee of IETS Import/Export Committee.

M paratuberculosis is not consistently removed from bovine embryos by washing (Rhode *et al* 1990) and this measure cannot be relied on to remove these bacteria from ovine and caprine embryos. However embryos from infected animals are unlikely to be infected.

The Australian National Johne's Disease Program accepts that the risk of transmission of ovine Johne's disease in semen or washed embryos is negligible. In the Australian Sheep Johne's Disease Market Assurance Program (May 1997) semen and washed embryos are listed as acceptable means for the introduction of stock into an assessed flock. This is also the recommendation in the Australian Johne's Disease Market Assurance Program for Goats (April 1999).

Testing and examination

Detection of infection in asymptomatic sheep is difficult. Faecal culture is specific, but is not reliable in small ruminants (Perez *et al* 1997). The CF test, ELISA and AGID are commonly used for serological diagnosis of infected animals. Of these, the absorbed ELISA is considered to be the most sensitive serological test. The sensitivity of the ELISA test in sheep ranges from 48.4% to 83% (Perez *et al* 1997; Gwordz *et al* 1997).

Vaccination

Effective treatment regimes for Johne's disease in small ruminants have not been published. Vaccination can be effective in reducing disease incidence but does not eliminate or prevent infection.

Pre-export quarantine

The extended duration of the incubation period for Johne's disease precludes any benefit which might arise from holding animals in quarantine for prolonged periods.

Measures for embryos

No risk management measures are required for embryos.

Measures for semen

Donors

EITHER showed no clinical symptoms of Johne's disease during the pre-collection period and during collection and gave a negative result to an approved absorbed enzyme-linked immunosorbent assay (ELISA) or an agar gel immunodiffusion test (AGID) for JD between 90 days before the first collection of semen and export

OR

have been kept in a flock or herd in which no clinical sign of paratuberculosis was officially reported during the 5 years immediately prior to collection.

6.5 Brucella ovis

Disease freedom of animals in country, zone or flock

The recommended requirements for country or zone freedom from *B ovis* are described in *Code* Article 3.3.2.1.

Washing embryos

Washing embryos may not remove *B ovis*. Wolfe *et al* (1988) failed to remove bacteria from ovine blastocysts after exposing them *in vitro* to *B ovis* then washing them ten times. Ovine embryos exposed *in vitro* to *B ovis* and washed ten times without antibiotics, transmitted infection to recipient ewes (Riddell *et al* 1990). *B ovis* is an IETS category 4 pathogen in sheep.

Testing and examination

The AGID, CF or ELISA using soluble surface antigens obtained from *B ovis* are the most widely used serological tests. A combination of AGID and ELISA is reported to give the highest sensitivity but the prescribed test for international trade is the CFT.

Measures for ovine embryos

No risk management measures are required for embryos.

Measures for ovine semen

Donors :

EITHER

have lived only in countries in which B ovis infection has not been reported

OR

have lived only in flocks/herds accredited *officially free* by the *Veterinary Administration* of the exporting country

OR

gave a negative result to a complement fixation test (CFT) or an approved absorbed enzyme-linked immunosorbent assay (ELISA) for *B ovis* between 90 days before the first collection of semen and export. There are no measures for goats.

6.6 Brucella melitensis

Disease freedom of animals in country, zone or flock

The recommended requirements for country or zone freedom from caprine and ovine brucellosis (excluding *B ovis*) are described in *Code* Article 3.3.2.1. Brucellosis caused by *B melitensis* is reported in France, Spain, Greece, Portugal and Italy.

Washing embryos

There is no information on the removal of *B melitensis* from the surface of embryos by washing or exposure to trypsin or antibiotics. *B ovis* appears to attach closely to the ZP of ovine blastocysts and embryos. Under experimental conditions *B ovis* was not removed after ten washings and treatment with penicillin and streptomycin (Wolfe *et al* 1988; Riddell *et al* 1990). *B abortus*, by contrast, was removed by washing and antibiotic treatment of infected ovine (and bovine) embryos (Riddell *et al* 1989b). These inconsistent results of embryo washing may be due to differences in the nature of bacterial cell surfaces. If so, *B melitensis*, a smooth form like *B abortus*, should be readily washed from ovine and caprine embryos.

The risk of transmission of *B melitensis* by embryo transfer has not been determined by the Research Subcommittee of IETS.

Testing and examination

The CFT and Rose Bengal plate agglutination tests using *B abortus* biovar 1 as antigen are prescribed tests for international trade. Both tests are considered to be too insensitive to detect individual animals infected with *B melitensis* (Blasco *et al* 1994). Latent or carrier infections of sheep occur and infected animals may be seronegative with these tests (Grillo *et al* 1997; Alton 1984). ELISA tests under development promise increased sensitivity (Bercovich *et al* 1998). Until these tests become commercially available, the use of both the CFT and Rose Bengal plate agglutination tests to screen flocks is recommended in the OIE *Manual*.

Vaccination

An attenuated live vaccine is available for use in endemic countries to protect animals from disease (Scharp *et al* 1999). However, the *Code* does not consider animals from herds containing vaccinated animals suitable for international trade into countries free from *B melitensis*.

Measures for embryos

Donors :

EITHER

have lived only in countries or zones which meet *Code* requirements for country freedom (Article 3.3.2.1.) OR

immediately prior to the *pre-collection* period, was part of a flock/herd officially free from *Br melitensis* infection (Article 3.3.2.2.) and gave a negative result to a complement fixation test (CFT) and a Rose Bengal plate agglutination test for *Br melitensis* infection on the same blood sample taken during the *pre-collection* period

AND

the embryos were collected, washed, processed and stored in accordance with OIE Animal Health *Code* (Appendix 4.2.3.3.).

Measures for semen

Donors:

EITHER

have lived only in countries or zones which meet *Code* requirements for country freedom (Article 3.3.2.1.) OR

immediately prior to the *pre-collection* period, was part of a flock/herd officially free from *Br melitensis* infection (Article 3.3.2.2.) and gave a negative result to a complement fixation test (CFT) and a Rose Bengal plate agglutination test for *Br melitensis* infection on the same blood sample taken during the *pre-collection* period.

6.7 Mycoplasma agalactiae (Contagious agalactia)

Disease freedom of animals in country, zone or flock

CA is widely distributed in the Mediterranean basin (Bergonier *et al* 1997). The *Code* does not provide recommendations for country or flock freedom for CA.

Washing embryos

The risk of transmission of *Mycoplasma agalactiae* has not been determined by the Research Subcommittee of IETS Import/Export Committee.

Testing and examination

The Code describes a CFT which is in current use for the serodiagnosis of contagious agalactia (Perreau 1976). An ELISA has been recently described that gives results which correlate well with those of the CFT, is specific for *M agalactiae* and is more sensitive than the CFT (Levisohn *et al* 1991). Several PCR assays for *M agalactiae* have been reported (Dedieu *et al* 1995; Tola *et al* 1997).

Vaccination

Killed vaccines are considered to lack efficacy in the field (Manual).

Measures for embryos

Donors must be free of clinical signs and have lived on premises in which contagious agalactia had not been diagnosed during the 6 months immediately prior to the *pre-collection period*. AND

the embryos were collected, washed, processed and stored in accordance with OIE Animal Health *Code* (Appendix 4.2.3.3.).

Measures for semen

Donors must be free of clinical signs and have lived on premises in which contagious agalactia had not been diagnosed during the 6 months immediately prior to the *pre-collection period*.

6.8 Maedi-visna virus

Disease freedom of animals in country, region or flock

The *Code* does not provide requirements for country/territory freedom. Houwers (1990) recommends certification of freedom from MVV of flocks of origin based on recent serological examination of the whole flock with negative results. The reporting of country status with respect to MV is unreliable (Brodie *et al* 1994; Constable *et al* 1996), so a requirement for country freedom is not considered to be a useful risk management option.

Studies have shown the average flock seroprevalence of MV ranged from 19% to 97% in Canada, the USA and some EU countries (Constable *et al* 1996; Lujan *et al* 1993; Houwers *et al* 1987).

MV accreditation schemes operate in the UK, Denmark and the Netherlands. Isolation and testing with removal of reactors in these flocks over prolonged periods provides assurance that animals are free from MV.

Washing

MVV did not transmit from infected sheep and goats through transfer of washed embryo (Dawson and Wilmot 1988; Young 1993). In spite of these findings, IETS (1998) regard this disease agent as Category 4 in sheep and unclassified in goats.

Testing and examination

Clinically normal infected animals may be detected by serology or virus isolation.

The period between exposure to virus and the detection of antibodies varies with the route of infection, form of exposure and breed of sheep. Seroconversion occurs from 4 to 6 weeks following experimental infection and antibody levels tend to stay relatively constant (Petursson 1990). The first appearance of antibodies following natural infection can range from 11 months to over 5 years (Houwers *et al* 1987). Persistent high antibody titres are usual in infected animals but disease in the absence of positive serology has been described (Houwers *et al*. 1987). A complicating factor is that significant viral antigenic variation can occur in MVV infected animal over time (Narayan *et al* 1977). Also, the serological response to MVV varies with age and breed of sheep (Constable *et al* 1996).

The AGID and ELISA are most commonly used serological tests (Simard and Briscoe 1990). The AGID is the prescribed test for international trade. The test is specific, reproducible and simple to set up, but interpretation of results requires experience and it is less sensitive than the ELISA (Rosati *et al* 1994; Saman *et al* 1999). ELISAs using both recombinant protein antigens and whole virus are of comparable specificity. While both the sensitivity and specificity of the ELISA depend on the quality of the antigen, tests using whole virus are generally regarded to be more sensitive than tests based on recombinant antigens (Pasick 1998; Rosati *et al* 1994). Recombinant MVV proteins can be used in ELISA tests for the serodiagnosis of both CAEV and MVV infections (Pasick 1998b; Saman *et al* 1999).

Seropositivity increases with age of sheep in all breed types. Snowder *et al* (1990) determined the average seroprevalence to be 11% at one year of age and 93% in sheep 7 years or older. Cutlip *et al* (1992) found that prevalence increased from 4% at less than 1 year to 34 % at 4 years, with variability associated with breed type.

In summary, the time required for seroconversion following infection can be relatively prolonged and unpredictable. An infected animal may give a negative result to single antibody test so more than one test over a period would be expected to increase the likelihood of detecting infected animals.

MV virus can be detected by virus isolation or nucleic acid detection methods. Even though virus cannot be recovered directly from tissue homogenates (Carey and Dalziel 1993), virus isolation can be attempted by co-cultivating monocytes from blood or milk or other tissues with ovine cell cultures (*Manual*). A PCR assay can detect *gag* DNA (provirus) in monocytes of sheep, especially during the early stages of infection.

This PCR has been used to detect infected sheep in the Dutch National MVV/CAEV control program (Wagter *et al* 1998).

Vaccination

There are no vaccines available.

Pre-export quarantine

Animals infected with MVV typically show a very long incubation period so that PEQ is impractical.

Measures for ovine embryos

Donors :

EITHER

immediately prior to embryo collection were part of an accredited MV free flock recognised by the *Veterinary Administration* of the exporting country

OR

immediately prior to embryo collection were part of a flock in which MV had not been diagnosed during the previous 3 years and during this 3 year period no contact with goats occurred and no animals were introduced from flocks with a lesser disease status

AND

gave a negative result to

either

an approved ELISA for MV antibodies on two blood samples collected 30 days apart during the *pre-collection* period, at collection or at autopsy

or

an approved virus isolation technique using white blood cells (mononuclear cells) or embryo collection fluids collected at embryo collection or at autopsy

or

an approved nucleic acid recognition test, eg. a PCR test on white blood cells (mononuclear cells) or embryo collection fluids collected at embryo collection or at autopsy

AND

the embryos were collected, washed, processed and stored in accordance with OIE Animal Health *Code* (Appendix 4.2.3.3.).

Measures for ovine semen

Donors :

EITHER

immediately prior to entry into the AI centre were part of an accredited MV free flock recognised by the *Veterinary Administration* of the exporting country and during this 3 year period no contact with goats occurred and no animals were introduced from flocks with a lesser disease status

OR

immediately prior to entry into the AI centre were part of a flock in which MV had not been diagnosed during the previous 3 years and during this 3 year period no contact with goats occurred and no animals were introduced from flocks with a lesser disease status

AND

gave a negative result to

either

an approved ELISA for MV antibodies on two blood samples collected 30 days apart during the *pre-collection* period, at the time of collection or at autopsy

or

an approved virus isolation technique using white blood cells (mononuclear cells) or semen collected during semen collection after the pre-collection period or autopsy or

an approved nucleic acid recognition test, eg. a PCR test on white blood cells (mononuclear cells) or semen collected during semen collection after the pre-collection period or autopsy.

6.9 Caprine arthritis-encephalitis virus

Disease freedom of animals in country, region or flock

The *Code* does not provide recommendations for country/territory freedom for CAE. OIE country status reports can be inconsistent with reports of this disease in the literature (Contreras *et al* 1998) and country freedom is not included as a risk management measure.

Washing

IETS (1998) classifies CAEV as Category 4.

Testing and examination

Clinically normal infected animals may be detected by serology or virus detection.

Ovine and caprine lentivirus infections are persistent, so that antibody detection is a valuable serological tool for identifying virus carriers. Recombinant MVV proteins can be used in ELISA tests for the serodiagnosis of both CAEV and MVV infections (Pasick 1998; Saman *et al* 1999).

Seroconversion following infection can be relatively prolonged and an infected animal may give a negative result to a single antibody test. More than one test over a period would be expected to increase the likelihood of detecting infected animals.

CAE virus can be detected by virus isolation or nucleic acid detection methods. Virus isolation can be attempted by co-cultivating monocytes from blood or milk or other tissues with ovine cell cultures (*Manual*).

Vaccination

There are no vaccines available.

Pre-export quarantine

Animals infected with CAEV typically show a very long incubation period so that PEQ is impractical.

Measures for caprine embryos

Donors :

EITHER

immediately prior to embryo collection were part of an accredited CAE free herd recognised by the *Veterinary Administration* of the exporting country

OR

immediately prior to embryo collection were part of a herd in which CAE had not been diagnosed during the previous 3 years and during this 3 year period no contact with goats or sheep occurred and no animals were introduced from herds or flocks with a lesser disease status

AND

gave a negative result to

either

an approved ELISA for CAE antibodies on two blood samples collected 30 days apart during the *pre-collection* period, at the time of collection or at autopsy

or

an approved virus isolation technique using white blood cells (mononuclear cells) or embryo collection fluids collected at embryo collection or at autopsy

or

an approved nucleic acid recognition test, eg. a PCR on white blood cells (mononuclear cells) or embryo collection fluids collected at embryo collection or autopsy

AND

the embryos were collected, washed, processed and stored in accordance with OIE Animal Health *Code* (Appendix 4.2.3.3.).

Measures for caprine semen

Donors :

EITHER

immediately prior to entry into the AI centre were part of an accredited CAE free herd recognised by the *Veterinary Administration* of the exporting country

OR

immediately prior to entry into the AI centre were part of a herd in which CAE had not been diagnosed during the previous 3 years and during this 3 year period no contact with sheep occurred and no animals were introduced from herds with a lesser disease status

AND

gave a negative result to

either

an approved ELISA for CAE antibodies on two blood samples collected 30 days apart during the *pre-collection* period

or

an approved virus isolation technique using white blood cells (mononuclear cells) or semen collected during semen collection after the pre-collection period or autopsy

or

an approved nucleic acid recognition test, eg. a PCR test on white blood cells (mononuclear cells) or semen collected during semen collection after the pre-collection period or autopsy.

6.10 *Mycoplasma capricolum* subsp. *capripneumoniae* (contagious caprine pleuropneumonia)

Disease freedom of animals in country, zone or flock

The *Code* recommends recognition of country freedom one year following a stamping-out policy. The *Code* also acknowledges that chronic infections can occur and Nicolet (1994) further states that asymptomatic carriers can excrete mycoplasmas. A stamping out policy without serological screening of flocks may not ensure freedom of infection with *Mycoplasma capricolum* subsp. *capripneumoniae*.

Washing

It appears highly likely that the washing procedures recommended by IETS will not clear caprine embryos of mycoplasmas (Philpott 1993). Bovine embryos exposed *in vitro* to *Mycoplasma bovis* and *M bovigenitalium* remained infected despite washing and exposure to antibiotic and trypsin (Riddell *et al.* 1989a). Similarly *Mycoplasma mycoides* subsp. *mycoides* remained attached to the ZP of caprine embryos despite standard washing procedures (Guerin *et al* 1997).

The risk of transmission of mycoplasmas by the transfer of caprine embryos has not been determined by the IETS.

Testing and examination

Serological tests are of limited value in detecting infected individuals. Available tests are not highly sensitive or specific and acute cases caused by Mccp rarely show positive titres to the organism before death (Muthomi and Rurangirwa 1983; MacOwen and Minnette 1977). Also, cross reactions can occur in sera taken from goats infected with other members of the "*M mycoides* cluster". Wherever possible paired serum samples collected 7-8 weeks apart should be tested. This allows for the development of antibodies within the maximum incubation period recorded (50 days). The CFT and indirect haemagglutination (IHA) tests are recommended in the OIE Manual and it is acknowledged that the CFT is more specific and less sensitive than the IHA test. A diagnostic method for CCPP based on PCR of the 16S rRNA genes from *Mccp* and restriction enzyme analysis of the PCR product has been developed (Ros Bascunana *et al* 1994) but this method is not practical for health certification purposes.

Vaccination

Numerous vaccines have been developed but their efficacy may be doubtful or imperfectly evaluated (Nicolet 1994). The role of vaccination remains controversial as a way of preventing mycoplasmosis.

Measures for caprine embryos

Donors had lived only in countries or zones which met *Code* requirements for country freedom (Article 3.3.6.2.)

AND

the embryos were collected, washed, processed and stored in accordance with OIE Animal Health *Code* (Appendix 4.2.3.3.).

Measures for caprine semen

Donors had lived only in countries or zones which met *Code* requirements for country freedom (Article 3.3.6.2.).

6.11 Chlamydia psittaci (enzootic abortion of ewes)

Disease freedom of animals in country, zone or flock

The *Code* provides recommendations for freedom from EAE infection for sheep flocks and goat herds. These recommendations include serological testing of a statistically valid sample of herds and flocks. This measure is considered unsuitable given the lack of specificity of available serological tests and the uncertain taxonomic relationship between chlamydial isolates.

Washing embryos

Embryos collected from EAE infected ewes and washed ten times according the recommendations of the IETS did not transmit infection to recipient ewes or progeny (Williams *et al* 1998). The Research

Subcommittee of IETS Import/Export Committee has placed *Chlamydia psittaci* into Category 4 for sheep.

Testing and examination

The CFT is considered the standard test for EAE serodiagnosis. A rise in antibody titre to *C. psittaci*, detected by complement fixation (CF), is common after abortion or stillbirth, but this does not occur in every case. *C psittaci* shares common antigens with *C pecorum* and some Gram-negative bacteria, so that the CF test is not wholly specific, nor does it distinguish between responses to vaccination and to infection. Alternative serological tests have been developed, for example a recently described cELISA (Gut-Zangger *et al* 1999), but none has been sufficiently appraised so far for field use.

Vaccination

Killed and live vaccines are available that reduce the incidence of abortion, but do not prevent infection.

Measures for embryos

Donors have lived only on premises in which enzootic abortion of ewes had not been diagnosed for 2 years AND

gave a negative result to a CFT test for EAE during the pre-collection period

AND

the embryos were collected, washed, processed and stored in accordance with OIE Animal Health *Code* (Appendix 4.2.3.3.).

Measures for semen

Donors have lived only on premises in which enzootic abortion of ewes had not been diagnosed for 2 years AND

gave a negative result to a CFT test for EAE during the pre-collection period.

6.12 Jaagsiekte

Disease freedom of animals in country, zone or flock

The *Code* has no recommendations for reducing the risk of introducing jaagsiekte via embryo transfer or artificial insemination and does not provide recommendations for country/territory freedom for jaagsiekte. OIE country status reports can be inconsistent with reports of this disease in the literature. This appears to be the case for Spain (Lujan *et al* 1993) and the USA (Kwang *et al* 1995). Country freedom is therefore not included as a risk management measure.

Infected individuals cannot be reliably detected so the presumption that donors do not carry the virus depends on flock freedom. The disease free period required is greater than the observed outside median incubation period (4 years). This degree of caution is warranted as a number of situations could mask expression of disease:

- the flock of origin comprises a resistant breed, or breeds,
- the flock is managed so that animals older than 3 years are culled, and
- sheep with signs of respiratory disease are culled without determination of the cause.

Washing

Several significant trials conducted in the UK demonstrated that transmission of the virus by the transfer of washed embryos did not occur (Parker *et al.* 1998). In one study, 38 of 51 progeny from jaagsiekte positive donors survived for at least 5 years without evidence of jaagsiekte in recipients or progeny. A

range of British breeds were represented in both the donor and recipient ewes. Recipients were obtained from separate flocks which had a long history of freedom from jaagsiekte. In a separate study, 4 of 5 progeny from uninfected donors mated to an infected ram survived for at least 5 years and did not develop jaagsiekte. The recipients and their progeny were kept in a closed, isolated jaagsiekte-free flock. These results have not, as yet, led to changes to the IETS (1998) categorisation and JV is still regarded as Category 3.

Testing and examination

Detection of subclinical infection is difficult and diagnosis is dependent on identifying animals in a flock with typical clinical and post mortem signs. Disease expression may not occur in flocks which do not contain animals older than 3 years. If few sheep from these flocks are necropsied and subjected to the histopathological examination required to detect early jaagsiekte the presence of the disease may not be recognised. Early characteristic histopathological changes due to JV were detected in the lungs as multiple, small, well circumscribed nodules of mainly type II secretory cells lining the alveoli (Hunter and Munroe 1983; OIE Manual). These changes were described in detail by Moulton (1990). Nodules were detected within 10-20 days following experimental inoculation of newborn lambs (Hecht *et al* 1996).

The value of serological testing for the detection of infected animals is unclear. An ELISA has been described using a recombinant protein component of the putative virus as the antigen (Kwang *et al* 1995). However, most other workers have concluded that a specific immune response against JSV in sheep and goats naturally affected by jaagsiekte does not occur (York *et al* 1992; Ortin *et al* 1998).

A number of other detection methods for JV have been recently reported. A competition radioimmunoassay (RIA) was used to detect JV antigen in tumor cell homogenates, lung fluid, and cell culture supernatant fluids in sheep with naturally occurring and experimentally-induced infection (Kajikawa *et al* 1990). Recent research has shown that JV DNA can be detected in tumours in lung tissue, lung secretions of JV infected sheep, lymphoid tissues and PBMC by PCR (Palmarini *et al* 1996, 1999). An exogenous virus-specific hemi-nested PCR was developed using primers in the 3' unique sequence (U3) region of JV, where major differences between endogenous and exogenous sequences exist. This test consistently detected JV in mediastinal lymph nodes draining the lungs. JV transcripts were also detected in spleen, thymus , bone marrow and peripheral blood mononuclear cells (Palmarini *et al* 1996). A kinetic study of JV infection in the mediastinal lymphocyte population of newborn lambs inoculated with JV found that JV proviral DNA could be detected as early as 7 days post inoculation although the proviral burden was much less than that detected in natural cases in adult animals (Holland *et al* 1999).

Vaccination

Vaccines are not available.

Pre-export quarantine

Animals with jaagsiekte typically show a very long incubation period so that PEQ is impractical.

Measures for ovine embryos

Donors :

EITHER

have only lived in flocks which include animals older than 5 years, and in which, as far as can be determined, after due enquiry and examination of official records, all animals remained free from jaagsiekte, based on the absence of clinical signs, for at least 5 years immediately prior to collection of embryos during which no animals were introduced from flocks with a lesser jaagsiekte status

OR

gave a negative result to a pathological examination or immune or nucleic acid test for jaagsiekte virus/viral components in lung and associated lymphoid tissues in accordance with procedures approved by the *Veterinary Administration* of the exporting country for the detection of jaagsiekte.

AND

the embryos were collected, washed, processed and stored in accordance with OIE Animal Health *Code* (Appendix 4.2.3.3.).

Measures for ovine semen

Donors

EITHER

have only lived in flocks which include animals older than 5 years, and in which, as far as can be determined, after due enquiry and examination of official records, all animals remained free from jaagsiekte, based on the absence of clinical signs, for at least 5 years immediately prior to collection of semen during which no animals were introduced from flocks with a lesser jaagsiekte status AND

gave a negative result to a pathological examination or immune or nucleic acid test for jaagsiekte virus/viral components in lung and associated lymphoid tissues in accordance with procedures approved by the *Veterinary Administration* of the exporting country for the detection of jaagsiekte.

Appendix 1

The following pathogens associated with disease in sheep and goats are not given detailed consideration in this risk analysis. Pathogens are grouped under headings which provide an explanation for their exclusion.

Group 1 The following diseases/ pathogens are not considered in this risk analysis as they are present in Australia and not subject to a *National control program*.

Actinomycosis	Listeriosis
Bacillus anthracis (anthrax)	Liver fluke
Clostridial diseases	Melioidosis
Botulism	Pasteurellosis
Campylobacter spp.	Pestivirus (Border disease virus)
Caseous lymphadenitis	Salmonella spp.
Coccidiosis	Sheep mange
Contagious opthalmia	Toxoplasmosis
Contagious pustular dermatitis	Akabane disease
Coxiella burnetii (Q Fever)	
Cryptosporidia spp.	
Dermatophilus congolensis	
Echinococcus granulosus (hydatidosis)	

Group 2 The following exotic¹⁸ diseases/pathogens are not considered in this risk analysis as transmission via transfer of ovine and caprine embryos or semen is regarded as highly unlikely.

Screwworm *Trichinella* spp. Borna disease Coenurosis Foot rot Sheep scab Vesicular stomatitis ¹⁹

Group 3 The following diseases/pathogens are not reported in the countries which are the subject of this IRA.

Rinderpest Pestes de petits ruminants Rift Valley fever Heartwater Wesselsbron disease

¹⁸ Diseases/ pathogens not present in Australia, or present but subject to a National control program

¹⁹ Vesicular stomatitis is a disease of horses, cattle and pigs. Even though sheep, goats and many other wild species as well as humans can be infected, the virus is not considered a hazard of sheep and goats in this IRA.

Appendix 2 Explanation of IETS categories

Category 1

Diseases or disease agents for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled²⁰ between collection and transfer.

Category 2

Diseases for which substantial evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled¹⁹ between collection and transfer, but for which additional transfers are required to verify existing data.

Category 3

Diseases or disease agents for which preliminary evidence indicates that the risk of transmission is negligible provided that the embryos are properly handled¹⁹ between collection and transfer, but for which additional *in vitro* and *in vivo* experimental data are required to substantiate the preliminary findings.

Category 4

Diseases or disease agents on which preliminary work has been conducted or is in progress.

Sheep and Goat diseases categorised by IETS (1998)

Category 1 Nil

Category 2 Bluetongue (sheep)

Category 3

Campylobacter fetus abortion (sheep) FMD (sheep and goats) Scrapie (sheep) Ovine pulmonary adenomatosis (Jaagsiekte)

Category 4

Ureaplasma/Mycoplasma spp. infection (goats) Maedi visna Scrapie (goats) Bluetongue (goats) CAE *Chlamydia psittaci* (sheep) *Brucella ovis* infection (sheep) Border disease (sheep) Bovine spongiform encephalopathy (goats)

²⁰ *IETS Manual*, 2nd edition (Recommendations for the sanitary handling of embryos).

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