Import Risk Analysis: Scrapie in sheep and goat germplasm

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Policy and Risk
MAF Biosecurity New Zealand

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Approved for general release

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TABLE OF CONTENTS

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1. EXECUTIVE SUMMARY

This analysis considers the risk of introduction of scrapie through the importation of sheep and goat germplasm (semen or embryos). The risk analysis was considered necessary because there have been significant scientific advances since the last scrapie risk analyses were conducted in the early 1990s.

The results of embryo transfer experiments conducted since 2001 were examined, as well as the available literature on the likelihood of scrapie agent being present in semen.

The very significant advances made in understanding of the genetic control of scrapie were evaluated for their applicability in managing risks.

Developments in ante-mortem testing for the presence of scrapie infection were considered and evaluated for incorporation into import programmes. The developments in rapid post-mortem diagnostic tests were not considered in this analysis.

The analysis concludes that the likelihood of scrapie being introduced by embryo transfer is extremely low and the likelihood of introduction by semen is very low. However, because the risk of exposure is assessed as high and consequences of introduction are also high, the analysis concludes that measures to manage the risks are warranted.

Various risk management options, including the application of the international standards recommended in the OIE's *Terrestrial Animal Health Code*, are considered.

The possible risks posed by the agents of so-called 'atypical' scrapie and bovine spongiform encephalopathy in sheep and goat germplasm are also assessed.

2. INTRODUCTION

New Zealand breeders of sheep and goats would benefit from having access to new breeds or improved bloodlines. However, because of major concerns over the possible introduction of scrapie, importations have been, to a great extent, restricted to those from Australia, one of only two countries currently recognised by New Zealand as scrapie-free (the other being South Africa).

The development of embryo transfer technology, along with early evidence that the technique provided a barrier against the transmission of scrapie, led to a small number of importations of germplasm from 1984 onward. These importations are summarised below (Table 1).

The purpose of this *Import Risk Analysis* is to re-assess the risk of introducing scrapie through importations of sheep and goat embryos and semen from countries other than Australia and South Africa.

Table 1: **Importations of sheep and goat germplasm into New Zealand from 1984.**

Country of origin	Date of import	Date of release	Breeds of sheep or goats
Denmark and Finland	April 1984	November 1990	Oxford Down, Finnish Landrace, Texel
Denmark and Finland	February 1986	November 1990	Texel, Oxford Down, Gotland Pelt, White Headed Marsh, Finnish Landrace
Zimbabwe	February 1986	April 1993	Angora goats, Boer goats
Zimbabwe	1989	1994	Karakul
Israel	1991	1994	Awassi
Sweden	1992	1996	East Friesian
South Africa	1995	1999	Angora goats
United Kingdom[1]	1997	Not released	Transgenic sheep
Singapore[2]	2002	Not released	Argali

1. The importers abandoned this project and all sheep were slaughtered while still in quarantine.
2. The imported semen proved to be sterile when inseminated into ewes in quarantine.

2.1. COMMODITY DEFINITION

The commodities under consideration are frozen semen and *in vivo* derived embryos from sheep (*Ovis aries*) and goats (*Capra hircus*). Semen and embryos are referred to collectively as germplasm. The commodities will be:

- Collected and processed at suitable collection centres and laboratories that have been approved for the purpose by the *Veterinary Authority*[1] of the exporting country.

- Processed, packaged and transported in accordance with the standards of OIE's *Terrestrial Animal Health Code* (OIE 2009).

2.2. SCOPE

This non-quantitative analysis is carried out in accordance with the MAF Biosecurity New Zealand policy that risk analyses should provide the relevant technical data on which Import Health Standards (IHSs) will be based. An IHS may be required for any commodity at the discretion of the Director General as defined in Section 22 of the Biosecurity Act of 1993.

This risk analysis is confined to scrapie, which is a naturally occurring transmissible spongiform encephalopathy of sheep and goats. Other diseases of these species have been dealt with elsewhere (MAF Biosecurity New Zealand 2005, 2008a).

2.3. METHODOLOGY

The methodology used in this risk analysis is described in MAF Biosecurity New Zealand's *Risk Analysis Procedures – Version 1* (MAF Biosecurity New Zealand 2006) and is consistent with the guidelines in the OIE's *Terrestrial Animal Health Code* (OIE 2009).

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

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[1] ***Veterinary Authority*:** The OIE defines this as "the Governmental Authority of a Member Country, comprising *veterinarians*, other professionals and para-professionals, having the responsibility and competence for ensuring or supervising the implementation of animal health and welfare measures, international veterinary certification and other standards and guidelines in the *Terrestrial Code* in the whole country." (OIE 2009)

Figure 1:   The risk analysis process
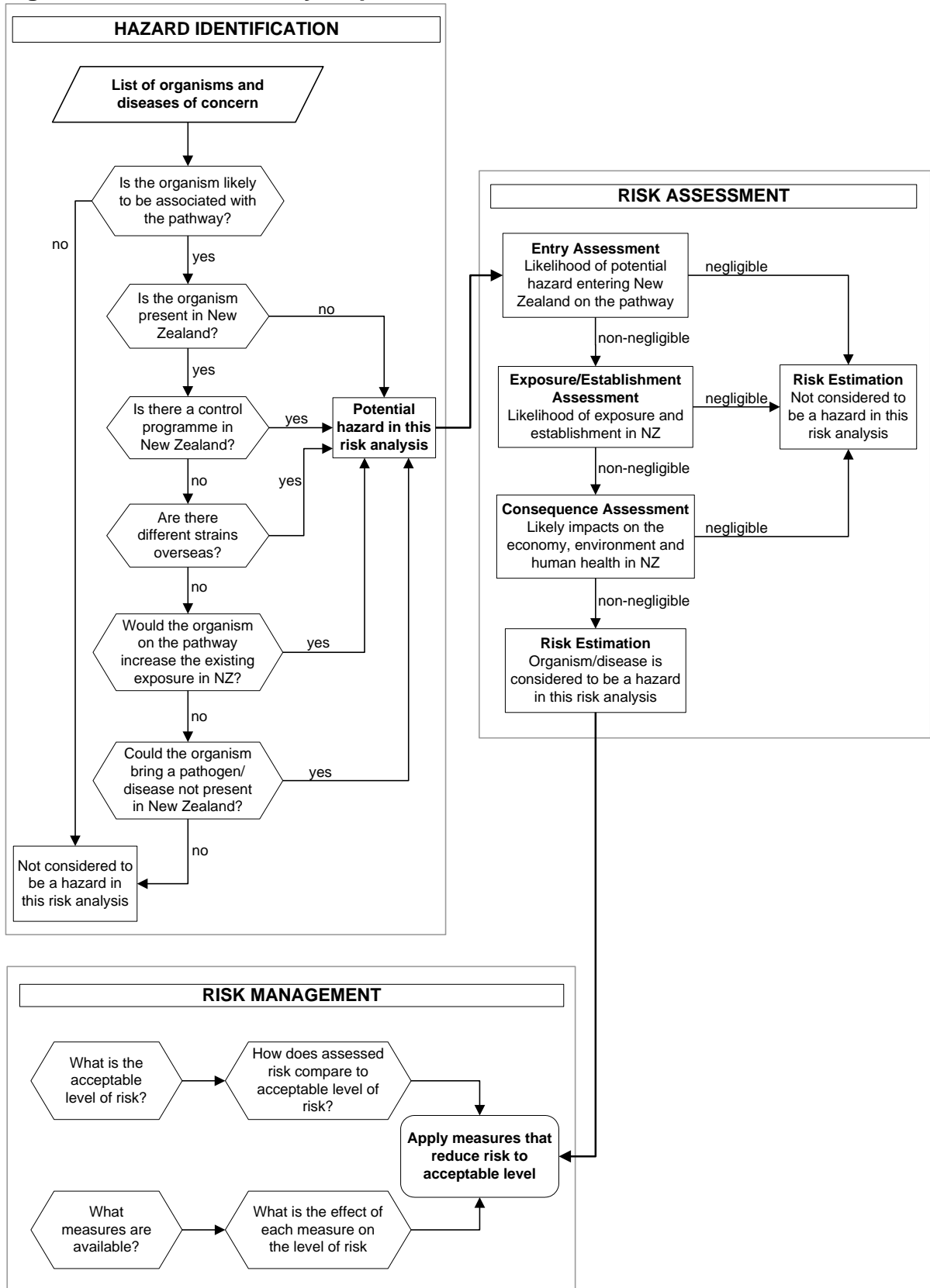
2.3.1. Hazard identification

The first step in the risk analysis process is *hazard identification*. This analysis covers a single pathogen only; the scrapie agent.

2.3.2. Risk assessment

In accordance with the risk analysis methodology used by MAF Biosecurity New Zealand, the potential hazard identified is subject to a risk assessment comprising;

a)   Entry assessment -   the likelihood of the organism being
                                          imported in the commodity.

b)   Exposure assessment -   the likelihood of animals or humans in New
                                          Zealand being exposed to the potential
                                          hazard.

c)   Consequence   the consequences of entry, establishment or
      assessment -   spread of the organism.

d)   Risk estimation -   a conclusion on the risk posed by the
                                          organism based on the release, exposure
                                          and consequence assessments. If the risk
                                          estimate is non-negligible, then the
                                          organism is classified as a hazard.

2.3.3. Risk management

For an organism classified as a hazard, an examination of risk management options is carried out. Where the OIE's *Terrestrial Animal Health Code* (the *Code)* lists recommendations for the management of a hazard, these are described alongside options of similar, lesser, or greater stringency where appropriate. In addition to the options presented, unrestricted entry or prohibition may also be considered for particular hazards.

Final recommendations for the appropriate sanitary measures to achieve the effective management of risks are not made in this document. These will be determined when an import health standard (IHS) is drafted.

As obliged under Article 3.1 of the *Agreement on the Application of Sanitary and Phytosanitary Measures* (the "SPS Agreement") (WTO 1995), measures adopted in an IHS will be based on international standards, guidelines and recommendations where they exist, except as otherwise provided for under Article 3.3 (where measures providing a higher level of protection than international standards can be applied if there is scientific justification, or if there is a level of protection that the member country considers is more appropriate following a risk assessment).

2.3.4. Risk communication

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

Following this period of public consultation on this draft document, a review of submissions will be produced and a decision-making committee will determine whether any changes need to be made to this draft risk analysis to make it final.

Following this process of consultation and review, the Animal Imports section of MAF Biosecurity New Zealand will decide on the appropriate combination of sanitary measures to ensure the effective management of identified risks. These will be presented in draft import health standards which are subsequently developed. Draft IHSs will also be released for a six-week period of stakeholder consultation and resulting stakeholder submissions will be reviewed before final IHSs are issued.

2.4. SPECIAL CONSIDERATIONS

Importation of semen and particularly embryos is generally accepted as being much safer than importing live animals.

In principle, semen or embryos should never be collected from animals that are showing clinical signs of an infectious disease and while, in this risk analysis, it is assumed that semen or embryos are collected only from animals that have been examined and found to be clinically healthy, in the case of a disease such as scrapie, which has an incubation period of many months, even years, such precaution provides little assurance.

Donors of germplasm should be kept on collection centres that meet the standards of the *Code (*OIE 2009). The methods of preparation of embryos and semen should follow the recommendations of the *Code*. Washing of embryos and

inclusion of trypsin in washing fluids influence the persistence of pathogens in prepared germplasm and the adherence of organisms to the *zona pellucida*.

New Zealand is one of the few countries that are widely recognised as being free from scrapie (MacDiarmid 1996). Nevertheless, the disease has occurred here twice in imported sheep, first in the 1950s and again during a second attempt at importation in the 1970s (Brash 1952a, Brash 1952b, Bruere 1985). Those experiences demonstrated the risk associated with attempting to import sheep without the imposition of appropriate measures aimed at excluding scrapie (MacDiarmid 1996). The occurrence of scrapie in imported sheep in quarantine in the 1970s resulted in a high level of concern amongst farmers and veterinarians and led to a lingering public controversy over MAF's importation policies (Adlam 1977, Bruere 1977a, Bruere 1977b, Bruere 1977c, O'Hara 1977, Bruere 1978a, Bruere 1978b, McPherson 1978, Bruere 1985, Annabell 1994, Bruere 2003).

In the early 1980s embryo transfer technology was used to import new sheep breeds from Scandinavia (Tervit et al 1986). This approach was considered to reduce significantly the likelihood of introducing scrapie (Bruere 1985, O'Hara 1987, MacDiarmid 1988). The Ministry of Agriculture developed a Scrapie Freedom Assurance Programme (SFAP) based on embryo transfer, bioassay in sentinel goats of mesenteric lymph node material from donor sheep, and a period of prolonged quarantine (3 to 5 years) for the embryo-derived offspring.

A method for quantitatively assessing the risk from such SFAPs was published between 1991 and 1996 (MacDiarmid 1991, MacDiarmid 1993, MacDiarmid 1996). The chain of safeguards used in the SFAPs has been accepted as providing adequate protection against the introduction of scrapie (MAF Biosecurity New Zealand 2008b).

In the years since the SFAPs were developed there have been significant advances in our understanding of scrapie, the results of new experiments have been published, and new diagnostic tools have been developed. It is, therefore, appropriate to re-assess the scrapie risk from the importation of sheep and goat germplasm.

3. RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Aetiological agent

Scrapie (or 'classical' scrapie) is one of a group of diseases known as the transmissible spongiform encephalopathies (TSE). It is an infectious disease of sheep and goats in which host genetic factors play a crucial role (Belt at al 1995). The aetiological agent of scrapie is widely, but not universally (Diringer 2001, Bradley and Verwoerd 2004, Manuelidis 2007, Hörnlimann et al 2007b, Jeffrey and González 2007), believed to be a prion. Prions are said to be agents which are clearly distinguishable from viruses, bacteria and other pathogens in that they are believed to be comprised solely of protein with no nucleic acid content (Hörnlimann et al 2007c). The prion is generally believed to be a misfolded isomer of PrP, a soluble protein found in cell membranes. The normal form is, by convention, referred to as PrP^c while the insoluble misfolded and proteinase resistant isomer is referred to as PrP^{sc}. According to the protein-only hypothesis, PrP^{sc} is the principal or sole component of the scrapie agent (Hörnlimann et al 2007b).

Scrapie is related to, but distinct from, 'atypical' scrapie (see Appendix 1) and bovine spongiform encephalopathy (BSE) (see Appendix 2).

3.1.2. OIE list

'Classical' scrapie is listed as reportable to the OIE. So-called 'atypical' scrapie is not reportable to the OIE because it is clinically, pathologically, biochemically and epidemiologically unrelated to 'classical' scrapie, may not be contagious and may be a spontaneous degenerative condition of older sheep (see Appendix 1).

3.1.3. New Zealand status

Exotic, unwanted organism.

3.1.4. Epidemiology

The epidemiology of scrapie has been reviewed extensively in recent years (Detwiler and Baylis 2003, Bradley and Verwoerd 2004, Hörnlimann, Riesner and Kretschmar 2007, Hunter 2007).

3.1.4.1. World distribution

The disease of sheep and goats which we call scrapie was first described in the literature in 1732 (Hörnlimann et al 2007b). It has an insidious onset and may escape notice in infected flocks. Scrapie has been found in many sheep-producing countries in the world and national claims to be free from the disease must be treated with scepticism. There is major difficulty in demonstrating national freedom from scrapie. Passive surveillance is widely considered to be inadequate, largely due to producer ignorance of the range of clinical signs and problems in reporting (Detwiler and Baylis 2003, Bradley and Verwoerd 2004). A number of countries claim to be free from scrapie, but reports in the scientific literature indicate otherwise. Examples are India (Zlotnik and Katyar 1961) and China (Feng et al 1987).

Australia, New Zealand (Detwiler and Baylis 2003, Bradley and Verwoerd 2004, Hörnlimann et al 2007c), Argentina (Schudel at al 1996, Bradley 2001, Hörnlimann et al 2007c, Secretaria Agricultura, Ganadera, Pesca Y Alimentacion 1997) and South Africa (MacDiarmid 1999, Bradley and Verwoerd 2004) are the only sheep-rearing countries widely accepted as free from scrapie.

The distribution of scrapie has historically been difficult to determine accurately because of lack of a preclinical test, clinical signs are variable, and farmers may be reluctant to report cases (Hoinville 1996). Even in countries where scrapie is endemic, the prevalence is seldom high. For example, the prevalence of scrapie in the European Union is low. In 2007, the European Union's surveillance programme found 2,253 (0.27%) of 828,644 sheep and 1,272 (0.46%) of 277,196 goats to be infected with scrapie (European Commission 2008). Estimates of scrapie prevalence may be made from a number of sources of data, but all have drawbacks (Gubbins 2008). By integrating a number of sources of data, Gubbins (2008) estimated that the prevalence of scrapie in Great Britain was between 0.33% and 2.06%. After consideration of various sampling and reporting biases, Baylis and colleagues estimated that the 'true' risk of scrapie in sheep of the highly susceptible VRQ/VRQ genotype in the UK lies in the range of 1,400 to 4,000 cases per annum per million of the genotype (see Table 2) (Baylis et al 2004). The prevalence of scrapie in the United States has been estimated to be around 0.07% (O'Rourke et al 2002).

The study by Gubbins (2008) indicated that a high proportion (55%) of sheep surviving long enough to die from scrapie die on farm, before the onset of obvious clinical signs, hence the incidence detected amongst fallen stock is likely to be higher than that detected through abattoir surveys.

Prevention of introduction is the key to scrapie freedom and requires restrictive import measures amongst which post-entry measures, such as prolonged

quarantine, have historically played a major role (Detwiler and Baylis 2003, Bradley and Verwoerd 2004, Hörnlimann et al 2007c). Rapid response upon detection following introduction, such as happened in New Zealand and Australia, is the key to success in scrapie eradication (Detwiler and Baylis 2003). National eradication of scrapie, once established, has not been achieved anywhere (Detwiler and Baylis 2003, Bradley and Verwoerd 2004, Doherr and Hunter 2007, Hörnlimann et al 2007c, Dawson et al 2008). Sheep infected with scrapie may incubate and spread the infection for several years before clinical signs develop and examination of sheep in flocks completely culled for scrapie has revealed a relatively high incidence of subclinical/preclinical infection (Georgsson et al 2008). Environmental contamination with scrapie agent, which may persist for several years, plays an important role in maintaining infection and hindering eradication once scrapie becomes established (Hoinville 1996, Doherr and Hunter 2007, Georgsson, Sigurdarson and Brown 2006).

3.1.4.2. The disease

Scrapie is an invariably-fatal neurological disease of adult sheep and goats. It is one of a group of diseases known as transmissible spongiform encephalopathies (TSEs). It is related to, but distinct from, bovine spongiform encephalopathy (BSE) (see Appendix 2).

Scrapie was not universally accepted to be a contagious disease until the 1970s; there were still many who considered it to be an inherited condition well into the 1960s (Parry 1983, Hoinville 1996). However, it is now universally accepted that scrapie is an infectious disease in which host genetic factors play a crucial role in influencing the disease phenotype (such as incidence, incubation time and pathogenesis (Belt at al 1995)).

The aetiological agent of scrapie is said to be a prion, an agent believed to be comprised solely of protein with no nucleic acid content (Hörnlimann et al 2007b). The prion is generally believed to be a misfolded isomer of a host-encoded cell surface glycoprotein called prion protein or PrP, a soluble protein found in cell membranes, and disease is associated with an accumulation of this insoluble, protease-resistant isomer called, by convention, PrP^{sc} while the normal isomer is known as PrP^{c} (Jeffrey and González 2007, Hörnlimann et al 2007b). The major genetic determinant of scrapie susceptibility is the PrP gene, *Prnp* (Hunter and Bossers 2007).

3.1.4.3. Clinical signs and course

The onset of scrapie is insidious. Behavioural changes may include increased excitability, nervousness or aggressiveness, particularly elicited by sudden noise or movement. Fine tremors of the head and neck and occasional convulsions may be seen. Lack of coordination of the limbs and abnormalities of gait are

common. Intense pruritus is common but is not observed in all cases (Aiello and Mays 1998).

The strain of scrapie agent influences the occurrence of disease in particular PrP genotypes (Hunter et al 1997, Jeffrey and González 2007, Reckzeh et al 2007). Pruritus and ataxia are often described but may be absent in some cases. Some sheep may die without overt clinical signs. Behavioural changes may be observed months before other obvious clinical signs (Jeffrey and González 2007).

The age at which clinical scrapie manifests in an infected flock is influenced by PrP genotype (Baylis et al 2002). The majority of clinical cases occur in sheep between 2 and 5 years of age (Hoinville 1996). For example, of 139 sheep with confirmed scrapie, the mean age was 3.0 years (Dennis et al 2009). Cases have been reported in animals as young as 12 months and as old as 11 years. Histopathological changes have been reported in the brains of 11 month-old lambs and infectivity has been reported in brains of lambs as young as 8 months of age (Hoinville 1996).

In a large study in Iceland, Georgsson and colleagues were able to detect scrapie infection in sheep as young as 4 months of age but there are reports in the literature of infection being detected in sheep even younger than this (Georgsson et al 2008). In a large study conducted in the UK by Baylis and colleagues, 80% of scrapie cases died aged 2 to 4 years. Only 3% of scrapie deaths were in animals older than 7 years (Baylis et al 2004).

3.1.4.4. Susceptibility

PrP genotype: Adult sheep as well as lambs are susceptible to infection with scrapie (Ryder et al 2004, Foster et al 2006a). There is a strong genetic component influencing the disease phenotype (such as incidence, incubation time, pathogenesis). The major genetic determinant of scrapie susceptibility is the PrP gene. In sheep, susceptibility to scrapie is very largely controlled by amino acid substitutions at three codons on the PrP gene (*Prnp*). The three codons are 136, 154 and 171.[2] The alleles at these codons are (Baylis et al 2004, Baylis and Goldmann 2004, Hunter and Bossers 2007):
  o Codon 136: alanine (A) or valine (V)
  o Codon 154: arginine (R) or histidine (H)
  o Codon 171: glutamine (Q), arginine (R) or histidine (H).

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[2] Several other polymorphisms may have some effect on susceptibility. Some have been shown to prolong incubation period (Lagreid et al 2008) but others occur at such a low frequency that it is difficult to determine their effect (Baylis and Goldmann 2004).

Of the 12 alleles of the PrP gene that are possible, only five are commonly seen. These are ARR, ARQ, VRQ, AHQ and ARH (Belt at al 1995). These five alleles combine to give the 15 genotypes commonly found in sheep (Baylis et al 2004, Baylis and Goldmann 2004).

The 15 PrP genotypes differ markedly in their susceptibility to scrapie (Baylis et al 2004, Baylis and Goldmann 2004). Sheep which are homozygous for glutamine at codon 171 (QQ_{171}) are most susceptible to scrapie. In Cheviot sheep, for example, $VV_{136}RR_{154}QQ_{171}$ (usually written VRQ/VRQ) are most susceptible (Hunter and Bossers 2007). In contrast, in Suffolk sheep, in which the VRQ allele is rare, ARQ/ARQ animals are most susceptible (Hunter and Bossers 2007).

The genotype most resistant to classical scrapie (and BSE[3]) is ARR/ARR[4] (Belt et al 1995, Andreoletti et al 2002, Baylis et al 2004, Baylis and Goldmann 2004, Hunter and Bossers 2007, Jeffrey and González 2007). Resistance of ARR/ARR genotype sheep to scrapie is not absolute: two, possibly three, cases of classical scrapie have been reported in sheep of this genotype (Groschup et al 2007, Goldmann 2008). Sheep carrying the AHQ allele are relatively resistant to scrapie and are unlikely to be inapparent carriers of infection (Andreoletti et al 2002, Thorgeirsdottir et al 2002, Goldmann 2008).

Sheep breeds may be broadly categorised on the basis of their *Prnp* alleles. There are the so-called "valine breeds", such as the Cheviot, Swaledale, Swifter, Romanov and Shetland that commonly have valine at codon 136 on at least one allele[5] and in which scrapie cases usually have VRQ on at least one allele. On the other hand, there are the "non-valine breeds" such as the Suffolk which rarely have valine at codon 136. VRQ/VRQ sheep are extremely susceptible to scrapie in the valine breeds while those with ARQ/ARQ are more resistant, at least in flocks in the United Kingdom.

In contrast, in non-valine breeds, ARQ/ARQ are susceptible (Hunter et al 1997, Hunter and Bossers 2007). In ARQ/ARQ sheep, incubation period may be considerably prolonged by polymorphisms at one or another of two other codons, 112 and 141 (Lagreid et al 2008).

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[3] Resistant to BSE in experimental challenge. There have been no naturally-acquired cases of BSE in sheep.
[4] Some experts consider that ARR/ARR homozygotes may not be the most resistant genotype and that heterozygotes such as ARQ/ARR may be more resistant. N Hunter personal communication with SC MacDiarmid, 2 July 2009.
[5] A useful convention when discussing differences in a single codon is to describe the allele as AXX/AXX etc, where X can mean any of the possibilities at the other codons.

In breeds which encode V at codon 136, VXX on one or both alleles is associated with a high scrapie risk and is apparently dominant. AXX/AXX can be susceptible to scrapie in some outbreaks if the codon 171 genotype is XXQ/XXQ, even in breeds encoding V_{136}. Sheep breeds in which V_{136} is absent or occurs at low frequency only have a high risk of scrapie in XXQ/XXQ genotypes (Hunter et al 1997).

Valine and non-valine breeds may respond differently to a particular strain of scrapie. In the Roslin Institute Neuropathogenesis Division's flock, Cheviots carrying VRQ are highly susceptible when inoculated with the SSBP/1 scrapie strain but are resistant when inoculated with CH1641. The converse applies in Cheviots carrying ARQ (Hunter and Bossers 2007). However, New Zealand-origin ARQ/ARQ Cheviots do succumb to the SSBP/1 scrapie strain, but with very long incubation periods.[6]

Belt and colleagues (1995) proposed that selection for the ARR allele could be useful in scrapie eradication programmes (Belt et al 1995).

Breed of sheep and strain of scrapie agent: The effect of the different alleles on scrapie susceptibility is complex. Both allelic frequency and the influence of the allele itself vary among sheep breeds (Vascellari et al 2005). The effect of PrP genotype can vary between breeds and between scrapie strains (Andreoletti et al 2000). Susceptibility to scrapie varies with the strain of the agent as well as the PrP genotype. In the United States, scrapie strains are described as valine-dependent or valine-independent. In the United States, scrapie usually, but not always, occurs in sheep carrying the ARQ allele. In contrast, in European sheep, scrapie more commonly occurs in sheep carrying the VXX allele. Over 90% of US scrapie cases are in sheep of the ARQ/ARQ genotype (Evoniuk et al 2005).

There are significant differences between breeds as to which genotypes are affected by scrapie. The Suffolk breed appears to lack the VRQ allele and the ARQ/ARQ genotype is the most susceptible. However, breed effects are not solely attributable to the presence or absence of the VRQ allele. In British Cheviot sheep both ARQ/ARQ and ARR/VRQ sheep appear resistant to scrapie. In French Romanov sheep the ARQ/ARR genotype is resistant while ARQ/ARQ sheep are susceptible. In British Texels, ARR/VRQ and ARQ/ARQ genotypes are both susceptible (Baylis et al 2004, Baylis and Goldmann 2004). ARQ/ARQ sheep in the UK appear less susceptible to scrapie than ARQ/ARQ sheep in some other countries (Goldmann 2008).

It is not known whether the differences in scrapie susceptibility observed between breeds are due to genetic differences in the sheep or to differences in scrapie strains circulating in the different breeds or different countries.

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[6] N Hunter personal communication with SC MacDiarmid, 2 July 2009.

Susceptibility may also be influenced by polymorphisms at codons other than 136, 154 and 171 (Baylis and Goldmann 2004) and dose of scrapie agent.

There is evidence that sheep of the same genotype and breed, in the same flock, may be susceptible to some strains of scrapie but resistant to others (Baylis et al 2004). Different strains of scrapie may have different incubation periods in sheep of the same genotype (Baylis et al 2004, Reckzeh et al 2007). Differences in scrapie susceptibility within the same genotype may be an effect of either the sheep breed or the strain of scrapie agent (Vascellari et al 2005).

Breeding for scrapie resistance: Baylis and co-workers used two extensive datasets to determine the risk of scrapie infection in British sheep of different genotypes. Risk was measured as 'reported cases per annum per million sheep' (RCAM) for each genotype (Baylis et al 2004, Baylis and Goldmann 2004). The study demonstrated that, in UK sheep, the greatest scrapie risk was in the ARQ/ARQ, ARH/VRQ and VRQ/VRQ genotypes (seeTable 2). The analysis also showed clearly the resistance conferred by the ARR allele. The ARR/ARR genotype was the only numerically significant genotype (around 21% of sheep in the UK) in which no scrapie cases were reported. The next most susceptible genotype after those with VRQ alleles was the ARQ/ARQ. This genotype is one of the most common in the UK (Baylis et al 2004, Baylis and Goldmann 2004). The genotype with the highest scrapie risk in the UK was the VRQ/VRQ. The second highest was the ARH/VRQ genotype (Baylis et al 2004, Baylis and Goldmann 2004).

Breeding programmes for scrapie resistance based on the polymorphisms at codons 136, 154 and 171 are possible. In the British National Scrapie Plan, genotypes were allocated risk scores from 1 to 5 (see Table 3) (Hunter and Bossers 2007). The National Scrapie Plan (NSP) was launched in the UK in July 2001[7]. Its aims were to reduce the risk of scrapie and BSE in the national flock through the genetic selection of purebred rams used in breeding. The NSP promoted the use of rams carrying the ARR allele and required slaughter or castration of rams carrying VRQ (Dawson and Del Rio Vilas 2008).Tested rams of certain genotypes could be purchased knowing that there was little likelihood of their developing scrapie. Similar programmes operate in a number of other countries (The Netherlands, France, Republic of Ireland, United States of America) (Dawson et al 2008).

By 2006, the majority of rams in use in British flocks had been genotyped (Dawson and Del Rio Vilas 2008).

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[7] With the accumulated evidence that BSE was not present in UK sheep and that there was, therefore, no public health benefit from the plan, the UK government ceased funding the NSP in October 2008.
http://www.defra.gov.uk/animalh/bse/othertses/scrapie/nsp.htm

The NSP has resulted in a reduction of scrapie-susceptible sheep in the UK and a corresponding increase in sheep of resistant genotypes (see Table 4). These changes signify a progressive reduction in the risk of classical scrapie affecting breeding rams and their progeny (Dawson and Del Rio Vilas 2008).

The incidence of scrapie in Great Britain, as demonstrated by surveys and surveillance, has fallen since the NSP was started (Dawson and Del Rio Vilas 2008, Dawson et al 2008).

The effect of genotype in goats: The situation in goats is more complex. While variation at codon 142 is associated with incubation time and codon 222 may be associated with susceptibility, not enough is known, however, to determine whether breeding for scrapie resistance is possible in goats in the same way that it is in sheep (Hunter and Bossers 2007, Dawson and Del Rio Vilas 2008, Barillet et al 2009, EFSA 2009).

PrP genotypes in New Zealand sheep: The 15 PrP genotypes commonly reported in other countries are all found in New Zealand sheep (Lee et al 2007, Hickford et al 2008).

Table 2: **Estimates of the number of cases of scrapie per million sheep per year of each genotype in the UK (Baylis et al 2004).**

Genotype	Cases per year (*n*)	Percentage of sheep	Cases per year per million (*n*)	95 % CI (lower–upper)
ARR/ARR	0	21·3	0	0·0–0·3
ARR/AHQ	0·3	5·6	0·3	0·0–1·6
ARR/ARQ	2·0	28·0	0·4	0·2–0·8
ARR/ARH	0	2·1	0	0·0–2·9
AHQ/AHQ	2·0	1·9	5·0	2·3–10·9
ARQ/AHQ	11·0	6·3	8·7	6·2–12·1
AHQ/ARH	0	0·3	0	0·0–20·6
ARH/ARH	0·7	1·6	2·0	0·5–7·3
ARQ/ARH	1·6	1·6	5·2	2·2–12·1
ARQ/ARQ	89·6	12·2	36·9	33·1–41·0
ARR/VRQ	12·0	9·6	6·3	4·5–8·6
AHQ/VRQ	0·3	2·5	0·7	0·0–3·7
ARQ/VRQ	264·0	5·9	225·4	214·7–236·0
ARH/VRQ	22·7	0·3	405·0	321·9–508·2
VRQ/VRQ	94·8	0·9	544·5	490·5–602·9

Table 3: **UK National Scrapie Plan PrP genotype risk groups (State Veterinary Service 2006, Dawson and Del Rio Vilas 2008).**

Genotype result	Type	Degree of resistance/susceptibility
ARR / ARR	1	Sheep that are genetically most resistant to scrapie.
ARR / AHQ ARR / ARH ARR / ARQ	2	Sheep that are genetically resistant to scrapie, but will need careful selection when used for further breeding.
AHQ / AHQ AHQ / ARH AHQ / ARQ ARH / ARH ARH / ARQ ARQ / ARQ	3	Sheep that genetically have little resistance to scrapie and will need careful selection when used for further breeding.
ARR / VRQ	4	Sheep that are genetically susceptible to scrapie and should not be used for breeding unless in the context of a controlled breeding programme approved by NSPAC.
AHQ / VRQ ARH / VRQ ARQ / VRQ VRQ / VRQ	5	Sheep that are highly susceptible to scrapie and should not be used for breeding.

Table 4: **Changes in PrP allele frequencies in the UK between 2002 and 2006**[8]

	2002	**2006**	**% change**
ARR	50.4	68.8	+ 36.5
AHQ	7.4	5.5	-25.7
ARH	9.9	5.6	-43.4
ARQ	29.2	18.9	-35.3
VRQ	3.0	1.2	-60.0

3.1.4.5. Transmission

The most common means by which scrapie is introduced into a previously uninfected flock is through the introduction of pre-clinically infected sheep (Hoinville 1996). The most common route of infection is believed to be orally and most transmission occurs at parturition or in the immediate post-partum period (Detwiler and Baylis 2003, Hörnlimann et al 2007c). Adult sheep as well as lambs are susceptible to infection with scrapie and horizontal transmission is the most important, if not the only, route of infection in both lambs and adult sheep (Ryder et al 2004, Evoniuk et al 2005). It has been demonstrated that scrapie may spread horizontally from infected adult sheep to susceptible adult sheep through prolonged contact, even in the absence of lambing (Foster et al 2006a).

The accumulation of the abnormal isoform (PrP^{sc}) of the normal cellular protein (PrP^{c}) correlates with the presence of infectivity (Andreoletti et al 2002). Scrapie infection of the neonate occurs at the time of, or soon after, birth and, in some flocks, PrP^{sc} is first detected in the lymphoid tissue of the lamb's digestive tract at 2 months of age (Konold et al 2008). As infection progresses, infectivity becomes widespread in the tissues (Jeffrey and González 2007). Infectivity has been detected by bioassay in the placenta, as has accumulation of PrP^{sc}. However, the placenta from an infected ewe may be positive (infective) at one gestation but negative at the next. PrP^{sc} accumulation in the placenta is linked to the genotype of the foetus (Andreoletti et al 2002).

The placenta is widely believed to be the main source of infection and milk, although able to transmit infection, is less important in the spread of the disease

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[8] Dr M Dawson, National Scrapie Plan Administration Centre, Worcester, UK. Personal communication with SC MacDiarmid, 29 April 2008.

(Konold et al 2008).[9] In lambs of the highly susceptible VRQ/VRQ genotype exposed to scrapie via milk from infected ewes, PrP^{sc} was detectable in lymphoid tissue at around 7 months of age. Susceptibility to scrapie infection is highest in the first year of life and decreases with age as involution of the Peyer's patches begins around 12 weeks of age. This involution is usually complete by 18 months of age (Konold et al 2008).

In scrapie cases in sheep of less-susceptible genotypes the distribution of PrP^{sc} is largely restricted to the gut-associated lymphatic tissue and the nervous system, so prions may not be secreted in the milk (Konold et al 2008).

Horizontal transmission of scrapie has been shown to occur in the absence of parturient ewes, which may shed the agent at the time of parturition. It is probable that exposure to faeces, urine or saliva, through shared food and water troughs, is the most likely route for this horizontal transmission when parturient ewes are not present (even though infectivity has not been detected in these excretions and secretions) (Konold et al 2008).

Infection of the lamb *in utero* is unlikely (Andreoletti et al 2002, Jeffrey and González 2007). No PrP^{sc} is found in foetuses, even when there are "massive levels" accumulated in the placenta. No PrP^{sc} is detectable in the tissues of newborn lambs of the highly susceptible VRQ/VRQ genotype born to infected ewes (Andreoletti et al 2002).

In a study of scrapie-infected flocks in Shetland, Jeffrey and colleagues (Jeffrey et al 2002) found no evidence to suggest that scrapie infection could be carried in, and transmitted by, sheep without them eventually becoming clinically affected by the disease.

Transmission via semen?:   Evidence from epidemiological studies and experimental matings of scrapie-affected rams suggests that scrapie is unlikely to be transmitted by semen (Wrathall 2000, Wang et al 2001, Wrathall et al 2008). Palmer (1959) failed to transmit scrapie by subcutaneous injection into lambs of semen from a clinically affected ram. The lambs were, however, only observed for 30 months post-inoculation and this observation period would now be considered less than ideal.

Bioassays in mice have failed to detect scrapie infectivity in testis, seminal vesicles and semen of affected rams (Hourrigan et al 1979, Hourrigan 1990, Hadlow 1991, Hourrigan and Klingsporn 1996). Hourrigan reported the failure to detect infectivity in semen samples from 21 cases of scrapie (Hourrigan 1990). The study by Hadlow et al (1980) showed that the distribution of scrapie infectivity in goats is essentially the same as in naturally-infected sheep.

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[9] Scrapie infectivity may be present in blood of pre-clinical cases (Houston et al 2008) and some experts believe that blood may be a source of perinatal infection. N Hunter personal communication with SC MacDiarmid, 2 July 2009.

Gatti and colleagues were unable to detect PrP^{sc} in seminal plasma of rams with scrapie, suggesting that infectivity was absent (Gatti et al 2002). The conclusions of this study have recently been confirmed by an experiment in which semen from infected rams was inoculated into scrapie-susceptible transgenic mice expressing the VRQ allele of the sheep prion gene (Sarradin et al 2008).

The transgenic mouse model used by Sarradin and colleagues (2008) has been shown to be capable of detecting very low levels of infectivity. The study reported by Sarradin and others (2008) demonstrated that scrapie was not transmitted by semen at any time during the incubation period of scrapie in four rams, even from one of the highly susceptible VRQ/VRQ genotype during the clinical stages of the disease.

Transmission via embryo transfer?:   There is good evidence that lambs do not become infected *in utero* (Andreoletti et al 2002, Jeffrey and González 2007). No PrP^{sc} is found in foetuses, even when there are "massive levels" accumulated in the placenta and none is detectable in the tissues of newborn lambs of the highly susceptible VRQ/VRQ genotype born to infected ewes (Andreoletti et al 2002). This provides assurance of the safety of pre-implantation embryos. If a foetus carried to term, in intimate association with the tissues of the dam, is unlikely to be infected, it is even less likely that a pre-implantation embryo, encased in its *zona pellucida*, will be infected. Over the years there have been a number of studies carried out to test this hypothesis.

In the 1980s Foote and colleagues reported that preimplantation ovine embryos could be transferred from experimentally infected ewes without transmitting scrapie to either the embryo recipient or the resulting offspring (Foote et al 1986, Foote et al 1993). Those early studies have been criticised because the genotypes of the sheep involved were not defined and some of the sheep may not have been of susceptible genotypes and the infection in the donors was experimentally induced, rather than acquired naturally (Wrathall et al 2008). However, the safety of embryo transfer has been confirmed by subsequent studies (Wang et al 2002, Foster et al 2006b, Low et al 2009).

The safety of embryo transfer has been confused by the apparent transmission of scrapie in a British study (Foster et al 1992, Foster et al 1996). However, this earlier British study, which produced results which could have been interpreted as indicating transmission by embryo transfer, has since been shown to have been compromised by lambing into a heavily contaminated environment. Two of the authors of these papers (Foster and Hunter) have stated; "Our early results of scrapie appearing in the [embryo derived] lambs were due to contaminated environment, I have absolutely no doubt of this"[10] and "The results of our earlier studies using embryo transfer were confounded by the incidence of natural

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[10] N Hunter, personal communication with SC MacDiarmid, 22 May 2008.

scrapie in our flock which manifested in some of the offspring."[11] The problem of lambing into the same scrapie-contaminated environment is alluded to in a later study by the same group (Foster et al 2006b).

The scrapie strain that infected the embryo-derived offspring in the earlier British study (Foster at al 1992, 1996) was different from the strain used to inoculate the embryo donors (Wrathall et al 2008). The early British study referred to above (Foster et al 1992, 1996) "contained flaws of design and implementation and it is most likely that there is no transmission of infectivity prior to parturition" (Jeffrey and Gonzalez 2007).

The criticism that the early US studies were flawed because experimentally infected donors were used, rather than ones with naturally acquired scrapie, has been addressed by a subsequent US study (Wang et al 2001). The criticism that the early US studies were flawed because the sheep were not appropriately genotyped was also addressed (Wang et al 2002).

A study was conducted by Wang and colleagues (2001) to examine the risk of scrapie transmission through embryos transferred from a naturally infected flock. The embryos were collected from a flock with a high natural incidence and were transferred into scrapie-free recipients held in a quarantine facility. Donor sheep were observed until death or a minimum of 60 months to determine whether they were infected or not. Scrapie-free recipients were kept in quarantine where they were observed for at least 60 months after embryo transfer. Embryo-derived offspring were maintained in the quarantine facility for at least 60 months or until death (Wang et al 2001).

Embryos with intact *zona pellucida* were washed 10 times in accordance with procedures recommended by the International Embryo Transfer Society. In total, 52 lambs were born from embryos collected from donors confirmed as scrapie-infected by histopathology and/or immunohistochemistry. Twenty two offspring survived 60 months without developing scrapie. Thirty one were derived from donors confirmed as infected by histopathology and 20 of these survived for 60 months. Twenty one offspring were derived from donors confirmed by immunohistochemistry to have scrapie and 13 survived for 60 months. Sixteen lambs were derived from donors confirmed as infected by both histopathology and immunohistochemistry and 11 of these survived for 60 months. Scrapie did not occur in the flock of recipient sheep (Wang et al 2001).

In a subsequent report, Wang and co-workers (2002) determined the genotype of the embryo-derived sheep reported in the study described above. They demonstrated that a high proportion (63%) of the embryo-derived sheep were of a genotype that is highly susceptible to scrapie (Wang et al 2002).

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[11] J Foster, personal communication with SC MacDiarmid, 22 May 2008.

These studies (Wang et al 2001, Wang et al 2002) provide good evidence that scrapie is not transmitted by transfer of *zona pellucida*-intact embryos washed according to International Embryo Transfer Society recommendations. Further evidence is provided in recent reports from Foster and colleagues (2006) and Low and colleagues (2009).

In 2006 Foster and colleagues reported a study in which a scrapie-free flock was established from embryos derived from a heavily infected flock. The embryo donors originated in a flock of Cheviot sheep in which 100% of those with the VRQ/VRQ genotype succumbed to scrapie and up to 60% of VRQ/ARQ sheep. The donors themselves were of the VRQ/ARR, VRQ/ARQ and VRQ/AHQ genotypes which are considered susceptible (VRQ/ARR) or highly susceptible (VRQ/ARQ and VRQ/AHQ) (See Table 2). Embryos were transferred into recipients of scrapie-resistant genotypes which were held in a scrapie-free establishment (Foster et al 2006b).

At the time the study was written up, there were 62 offspring of various PrP genotypes still alive in the scrapie-free flock. Approximately 37% (23) of these were of the highly susceptible VRQ/VRQ genotype and ranged from 71 to 107 months of age. (The mean age of natural scrapie cases in VRQ/VRQ sheep in the donor flock was 48 months.) A further 22 offspring were of susceptible genotypes and survived for more than 69 months. Tonsil biopsies were taken from a sample of the embryo-derived sheep and tested by immunohistochemistry for the presence of PrP^{sc}, with negative results. As a result of the study summarised here, Foster and colleagues concluded that it is highly unlikely that scrapie can be transmitted by embryo transfer (Foster et al 2006b).

The most recent study reported is that of Low and colleagues (Low et al 2009) in which embryos were collected from sheep in a naturally infected flock in which 56% of sheep of the ARQ/ARQ genotype were recorded as dying from scrapie. Embryos were transferred into recipients and the resulting offspring raised in a secure scrapie-free establishment. Donor ewes were either showing clinical scrapie at the time of embryo collection or developed signs after collection. Thirty nine lambs of the susceptible ARQ/ARQ genotype (see Table 2) resulted from the embryo transfers. Of these, 28 survived to the end point of the study at 5 years of age. All the sheep derived from embryo transfer were examined post-mortem for histological or immunohistochemical evidence of scrapie, with negative results.

The safety of goat embryos is supported by the study of Foster et al (1999).

It is clear that it is highly unlikely that scrapie can be transmitted by transfer of embryos collected and processed according to the recommendations of the International Embryo Transfer Society.

3.1.5. Hazard identification conclusion

Even though it is highly improbable that scrapie agent is likely to be associated with the commodities in question, New Zealand's historical experiences with scrapie require that it be considered a potential hazard when importing sheep and goat germplasm.

3.2. RISK ASSESSMENT

3.2.1. Entry assessment

Very few countries can plausibly claim to be free from scrapie. Even though the incidence of scrapie is very low in most countries where the disease is endemic, New Zealand's past experiences with importations of infected sheep indicate that there is a risk that the disease could be present in flocks from which germplasm might be sourced.

While there is very compelling evidence that scrapie is unlikely to be transmitted by embryo transfer, the possibility cannot be entirely ruled out. The likelihood of scrapie being introduced in imported embryos is therefore assessed as non-negligible; that is, extremely low.

While there is also strong evidence that scrapie is unlikely to be transmitted by semen, the possibility cannot be entirely ruled out. The likelihood of scrapie being introduced in imported semen is therefore assessed as non-negligible; that is, very low.

3.2.2. Exposure assessment

Imported germplasm of sheep and goats would come into intimate contact with potentially-susceptible sheep and goats in New Zealand, thus exposing them to any scrapie agent which might be present in the commodity. The likelihood of exposure, should scrapie agent enter New Zealand in the commodity, is assessed as moderately high.

3.2.3. Consequence assessment

It is extremely difficult to estimate the costs of introducing scrapie into New Zealand. Since the BSE epidemic of the 1990s, all the transmissible spongiform encephalopathies of animals have acquired a sinister reputation which has led to extreme reactions from consumers and major impacts on international trade. It is conceivable that there could be repercussions out of proportion to the actual effects on livestock production should scrapie occur here.

Following the diagnosis of scrapie in an imported ram near Ashburton in 1952, inadequate response measures led to a second occurrence in Southland in 1954. The successful eradication response to this second incident involved the slaughter of 4,339 sheep and the quarantine of 191 farms for 3 years (Bruere 1985). It is difficult to assess the consequences should scrapie become established in the New Zealand sheep population. One should exercise caution in extrapolating between countries with different animal husbandry systems. Nevertheless, it may be relevant that in Cyprus, where scrapie was first detected in 1985, more than 9% of flocks became infected within 15 years, despite control efforts (Gravenor et al 2004). Should scrapie be introduced into New Zealand, Bruere (1985) suggested that 1,000 flocks could become infected within 50 years. However, MacDiarmid (1996) suggested that this estimate could be considered conservative, given the rapidity with which newly-introduced sheep breeds were distributed around New Zealand following the importations of the early 1990s.

The long incubation period of scrapie and the lack of pre-clinical *in vivo* diagnostic tests mean that eradication has seldom been successful. The few successful attempts to eradicate the disease occurred in New Zealand and Australia, before the disease became established, and in South Africa, where it was eradicated before it became widely disseminated through the national flock (MacDiarmid 1996). (The only other sheep-producing country widely accepted as free from scrapie, Argentina, has never experienced an outbreak of the disease (Secretaria Agricultura, Ganadera, Pesca Y Alimentacion 1997)).

If scrapie were to spread within New Zealand flocks to an extent similar to that in northern hemisphere flocks (probably unlikely because of our more extensive sheep-rearing systems), losses could be high in some flocks. In northern hemisphere flocks infected with scrapie, annual mortalities are said to typically range from 3 to 5% but in some cases may be greater than 10 or 20% (MacDiarmid 1996, Smit et al 2002, Hörnlimann et al 2007c).[12] In the United States in 2001 it was estimated that scrapie costs the relatively small[13] US sheep industry between US$20 and US$25 million annually (Smit et al 2002).

The introduction of scrapie into New Zealand would adversely affect exports of live sheep and goats and, possibly, their germplasm, at least to countries claiming freedom from the disease (MacDiarmid 1996). Smit and colleagues

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[12] These figures may be misleadingly high. In a recent well-conducted study in Great Britain, Ortiz-Pelaez and Del Rio Vilas (2009) determined the within-flock prevalence of scrapie to be 0.65% (95% confidence intervals 0.55-0.75%)
[13] Around 5.7 million sheep.
http://www.oie.int/wahis/public.php?page=country_population&year=2008&selected_species=15

consider that the presence of scrapie in the US prevents the export of breeding stock, semen and embryos to many countries (Smit et al 2002).

It has been argued that the presence of scrapie in New Zealand could adversely affect exports of sheep meat to some countries (Bruere 1985) but, given that most sheep producing countries except New Zealand, Australia, Argentina and South Africa are generally regarded as infected with the disease, major barriers to trade are unlikely. The presence of scrapie would, however, probably result in changes to the way that ovine offals are disposed of, as they are currently rendered into meat-and-bone meal. The OIE's *Terrestrial Animal Health Code* (OIE 2009) recommends that meat-and-bone meal containing any sheep or goat protein which originates from a country not considered free from scrapie should not be traded internationally for feeding to ruminants.

It is likely that the industry most adversely affected by the introduction of scrapie would be the biopharmaceutical industry. The BSE epidemic in Europe and North America has led to many pharmaceutical manufacturers sourcing certain raw materials from New Zealand. In 1996, MacDiarmid stated that the rapidly growing biopharmaceutical industry was worth around $150 million per annum and that industry representatives believed that the introduction of scrapie could destroy the industry (MacDiarmid 1996). More recently, the biopharmaceutical industry has been estimated to be worth around $500 million per annum.[14]

Scrapie is not considered to be a risk to **human health** (Hörnlimann et al 2007c). A number of studies have failed to demonstrate any human health risk from scrapie (Brown et al 1987, Harries Jones et al 1988, Kondo and Kuriowa 1982, Van Dijn et al 1998, Budka 2007, Zerr and Poser 2007).

There would be no effect on **native species** from the introduction of scrapie. Natural transmission of scrapie has been observed only to sheep, goats and moufflon. The disease may be transmitted, with varying success rates, by experimental inoculation into some laboratory species (mice, hamsters) but not others (guinea pigs, rabbits) (Barlow and Rennie 1976, Vorberg et al 2003). Cats and dogs do not appear to be susceptible (Hamir et al 2002, Wu et al 2006). The PrP-like proteins of birds, reptiles, amphibians and fish exhibit a low sequence homology with mammalian PrP (that is, they are very different from mammalian PrP) and susceptibility of non-mammalian species to scrapie is extremely unlikely (Baylis and Goldmann 2004, Ji, Zhang and Chen 2007).

The consequences of scrapie introduction into New Zealand are assessed as high.

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[14] Lachlan McIntyre, Senior Adviser Surveillance, MAF Biosecurity New Zealand. Personal communication with SC MacDiarmid, 23 June 2009.

3.2.4. Risk estimation

The likelihood of entry of scrapie agent in the commodity is assessed as extremely low to negligible. However, the likelihood of exposure following entry is assessed as moderately high and the consequences are assessed as high. Risk management measures are required.

3.3. RISK MANAGEMENT

The history of scrapie introductions into New Zealand and other countries illustrates the risk of introducing scrapie without the imposition of appropriate safeguards. While some experts recommend that imports should be limited to animals from closed certified scrapie-free flocks and scrapie-free countries, and long-term monitoring of imported animals is important to avoid introducing scrapie (Doherr and Hunter 2007), New Zealand experience in the past 20 years has shown that safe importations can be made using a combination of risk management measures. Developments since the 1990s have led to a better understanding of the risks of introducing scrapie in germplasm and suggest that fewer safeguards may provide assurances at least equal to those provided by historical Scrapie Freedom Assurance Programmes (SFAPs).

3.3.1. Importation from countries free from scrapie

Certification of country freedom, without a confirmatory evaluation, is not sufficient. A number of countries report to the OIE that scrapie has never occurred, and yet there are reports of scrapie occurrence in scientific journals. Examples are India (Zlotnik and Katyar 1961) and China (Feng et al 1987).

Historically, New Zealand has recognised Australia as free from scrapie (MacDiarmid 1996) and importations of sheep and goats, and their germplasm, have been made without any specific scrapie-related safeguards. Following an assessment visit in April 1999 and stakeholder consultation, New Zealand recognised South Africa as free from scrapie (Agricultural Security Consultation Committee 1999, MAF Biosecurity Authority 1999).

There is compelling evidence that Argentina's claim to be free from scrapie (Schudel at al 1996, Bradley 2001, Hörnlimann et al 2007c) is at least as good as New Zealand's. Unlike New Zealand, Australia and South Africa, scrapie has never occurred in Argentina. An extensive assessment of scrapie risk factors in Argentina conducted in 1997 and updated in 2009 (Secretaria Agricultura, Ganadera, Pesca Y Alimentacion 1997, SENASA 2009), documented a history of sheep importations similar to New Zealand's, an extensive grazing system without the use of concentrates, a ban on the feeding of ruminant protein to ruminants, and awareness and surveillance programmes at least equal to, if not better than, New Zealand's. Argentina meets the criteria prescribed in the *Code* (OIE 2009) to be recognised as scrapie free and should be accepted as such by New Zealand.

The *Code* outlines the requirements for determining the scrapie status of a country. Scrapie status can be determined on the basis of a risk assessment, an on-going awareness programme and an extensive surveillance system (OIE

2009). Within this context, a country or an establishment (a farm) may be determined to be scrapie free. Very few countries fulfil the OIE's requirements to be considered free from scrapie. It is possible that, within some developed countries, there would be establishments that would meet the OIE's criteria for scrapie freedom.

Sheep and goat germplasm could be imported with little risk from countries or establishments that could be verified as meeting the OIE's criteria for scrapie freedom.

3.3.2. Restrict imports to certain PrP genotypes

It has been shown (see Table 2) that there is very little likelihood of scrapie occurring in sheep of the ARR/ARR PrP genotype. Putting aside for the moment issues of the safety of semen and embryos, regardless of other possible measures, sheep semen could be imported with virtually no risk if importations were restricted to rams of this genotype.

Similarly, importation of embryos could be restricted to those derived from ARR/ARR ewes mated to ARR/ARR rams. However, such a restriction on embryos is unwarranted, as the safety of embryos **has** been clearly demonstrated, and would probably seldom be practical.

It has been shown that tissue distribution of PrP^{sc} is limited in sheep carrying the ARR allele (Evoniuk et al 2005). PrP^{sc} accumulation in the placenta is controlled by the genotype of the foetus and is found only when the lamb is of a susceptible genotype (Andreoletti et al 2002). It has been shown that scrapie-infected ewes inseminated with semen from rams of the resistant ARR/ARR genotype produced heterozygous ARR lambs and no PrP^{sc} accumulated in the placenta (Andreoletti et al 2002).

Thus, importation of embryos could be restricted to those produced from ewes of any PrP genotype inseminated with semen from rams of the ARR/ARR genotype. Thus, even if the donor ewe were infected with scrapie, it would not be possible for the offspring to be infected *in utero*, and pre-implantation embryos would pose no risk of scrapie introduction.

Because not enough is known to determine whether breeding for scrapie resistance is possible in goats in the same way that it is in sheep (Hunter and Bossers 2007, Dawson and Del Rio Vilas 2008, Barillet et al 2009), it is not possible at this time to propose restrictions on germplasm derived from any particular genotype.

3.3.3. Ante-mortem tests for scrapie

Historically, the absence of an ante-mortem diagnostic test to detect infected animals has hampered attempts to manage the risks of spreading scrapie (Hoinville 1996). The SFAPs under which imports of sheep and goat germplasm have been made since 1989 (see Table 1) have used a bioassay in goats to screen donor animals for scrapie infection (MacDiarmid 1996). There are important drawbacks in the use of bioassays; the main one being the length of time required. In the New Zealand SFAPs the sentinel goats which were inoculated with lymph node material collected from donor animals were observed for a period of 3 years before the presence of scrapie could be ruled out with confidence.

Since New Zealand's SFAPs were designed in the late 1980s there have been significant developments in testing for the presence of scrapie by examining biopsies of lymphoid tissue for the presence of PrP^{sc}. Bioassays and these newer tests are discussed below.

3.3.3.1. Bioassays

Intracerebral bioassays, in which mesenteric lymph node material is inoculated into sentinel goats, have been used in New Zealand's SFAPs to screen imported sheep for pre-clinical scrapie since 1990 (MacDiarmid 1990). In 1990, a review of the literature and extensive consultation with international scrapie experts led to the conclusion that bioassay, by intracerebral inoculation of lymph node material into goats, was the best and most sensitive technique for detection of scrapie in sheep used as donors of embryos or semen (MacDiarmid 1990). The technique of intracerebral inoculation has been described more recently (Hamir et al 2008).

In a comparison of transmission of scrapie to sheep by the intracerebral and intralingual routes, there was no significant difference in time from inoculation to development of scrapie; around 18 months in both cases. Inoculation by the intralingual route is less invasive and has lower risk of adverse side effects (Hamir et al 2008).

Scrapie infectivity and PrP^{sc} is found in spleen and lymph nodes of sheep with scrapie (Van Keulen et al 1996) and is detectable in lymphoid tissue well before clinical disease occurs (O'Rourke et al 1998). Infectivity first accumulates in the lymphoid tissue of the alimentary tract (Van Keulen, Vromans and Van Zijderveld 2002). In the first major study of the pathogenesis of scrapie, Hadlow and co-workers (1982) reported that scrapie infectivity is detectable in mesenteric lymph nodes as early as 10-14 months of age, well before it is found in the central nervous system. More recently, PrP^{sc}, which is commonly accepted as an indicator of scrapie infectivity, has been reported as being detectable in gut-

associated lymphoid tissues, such as mesenteric lymph nodes, in lambs as young as 2 months of age (Van Keulen, Vromans and Van Zijderveld 2002).

Distribution of scrapie infectivity in non-neural tissues of goats is essentially the same as in sheep (Hadlow et al 1980).

Intracerebral inoculations of goats with scrapie always results in disease in all animals and incubation periods are relatively short (Goldmann 2008).[15] Thus bioassay of lymphoid tissue into sentinel goats, as used in New Zealand's SFAPs, is a sensitive tool for detecting preclinical infection in sheep. PrP^{sc} was detected in the mesenteric lymph nodes of 54 of 55 (98%) sheep with scrapie (Van Keulen et al 1996). However, in sheep of the partially scrapie-resistant genotype VRQ/ARR, scrapie infectivity is not found in the lymphoid tissues in preclinical infections (Van Keulen, Vromans and Van Zijderveld 2002) and so bioassay would probably fail to detect that donors of this genotype were infected.

MacDiarmid (1990) stated that adverse effects of intracerebral inoculation are remarkably rare and deaths attributable to the technique are rare. However, mishaps have occurred when the technique has been applied in New Zealand and one could question whether the technique would still be acceptable, given the current greater awareness of animal welfare issues.

3.3.3.2.   Detection of PrP^{sc} in peripheral lymphoid tissues

Scrapie-specific accumulations of PrP are detectable in the lymphoid tissues of scrapie infected sheep before they can be detected in the central nervous system (Reckzeh et al 2007) and so demonstration of PrP^{sc} in tissues of the lymphoreticular system can be used to detect preclinical cases of scrapie (Jeffrey et al 2002). Biopsy of accessible lymphoid tissue such as tonsil, third eyelid or recto-anal mucosa-associated lymphoid tissue (RAMALT) followed by immunostaining for PrP offers the possibility of preclinical diagnosis of scrapie for single suspect cases or small groups. However, costs, practicality and welfare concerns inhibit its widespread use at flock level (Dawson et al 2008). The ability of this technique to detect scrapie infection is influenced by the genotype of the sheep and this may also limit its applicability (Hörnlimann et al 2007a). Biopsy of lymphoid tissues is unlikely to be sufficiently reliable to exclude all infected sheep (Hörnlimann et al 2007c).

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[15] Goldmann's 2008 review cites studies by Pattison et al from the 1960s as the source of this conclusion. Those studies predate accurate diagnostic tests, particularly for animals with prolonged incubation periods. In goat-to-goat intracerebral challenges in progress at USDA Pullman, incubation periods exceeding 1,000 days have been observed in inoculated goats with certain PrP polymorphisms. K O'Rourke, personal communication with SC MacDiarmid, 8 July 2009.

***Tonsil biopsies*:**   The possibility of developing tests based on detection of PrP^{sc} in peripheral lymphoid tissues was raised in 1991 when Japanese researchers demonstrated that PrP^{sc} could be detected in lymph nodes of scrapie-infected sheep several months before the development of clinical signs (Ikegami et al 1991). The practicability of such tests was demonstrated in 1996 when Schreuder and colleagues reported that immunohistochemistry on lymphoid tissue obtained from sheep tonsil biopsies was able to detect PrP^{sc} at less than halfway through the incubation period of scrapie., approximately one year before onset of clinical disease (Van Keulen et al 1996, Schreuder et al 1996). PrP^{sc} was detected in the tonsil of 54 of 55 (98%) sheep with scrapie (Van Keulen et al 1996). However, PrP^{sc} accumulation in palatine tissue is variable in natural scrapie. In some infected sheep, PrP^{sc} was detected in CNS but not tonsil tissue (Shimada et al 2005). This results from differences in PrP genotype, as the studies of Van Keulen and colleagues showed that PrP^{sc} was not detected in the tonsil tissue of infected sheep of the partly resistant VRQ/ARR genotype (Van Keulen et al 1996).

Further studies have confirmed that PrP^{sc} can be readily detected in the tonsillar lymphoid tissue of sheep less than half-way through the incubation period of scrapie. In sheep of the highly susceptible VRQ/VRQ genotype, this means PrP^{sc} is detectable at around 7 to 8 months of age (Schreuder et al 1998, Jeffrey et al 2001). Accumulation of PrP^{sc} in lymphoid tissues occurs later in sheep of less susceptible genotypes (Jeffrey et al 2001). In sheep of the less-susceptible VRQ/ARQ genotype, which have a longer incubation period, PrP^{sc} is detectable at 14 to 16 months of age (Schreuder et al 1998). Tonsillar biopsies can detect PrP^{sc} at least a year before the development of clinical signs of scrapie (Jeffrey et al 2001).

Tonsil biopsies are not without some adverse effects. General anaesthesia is required and, as a result of the biopsy injury, the animals stop feeding for one or two days and a few animals (perhaps 1%) may die.[16] These adverse effects are not seen when RAMALT (see below) is the tissue biopsied.

It should be noted that a negative tonsillar biopsy does not mean that a sheep is free from scrapie infection. In sheep of the semi-resistant VRQ/ARR genotype, tonsillar biopsies may be negative despite the sheep exhibiting clinical disease and having histopathologically confirmed scrapie (Schreuder et al 1998). Nevertheless, tonsillar biopsies of a representative sample of susceptible sheep may be a more accurate method of determining a flock's scrapie-free status than other methods, such as farmer's assurances on the absence of clinical signs, or monitoring scrapie status by examining samples of brains collected at slaughter (Schreuder et al 1998).

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[16] Lorenzo González, Veterinary Laboratories Agency, Lasswade, Scotland. Personal communication with SC MacDiarmid, 24 June 2009.

Third eyelid biopsies: Biopsies of third eyelid are useful for diagnosing preclinical scrapie infections (Vargas et al 2006). The technique was developed by O'Rourke and co-workers who, in 1998, reported that an immunohistochemistry assay on lymphoid tissue associated with the third eyelid (nictitating membrane) of sheep provided a practical method for the early detection of scrapie (O'Rourke et al 1998). As is the case with tonsil biopsies, PrP^{sc} is detectable in lymphoid tissue of the third eyelid of sheep well before clinical signs of scrapie develop (O'Rourke et al 1998, O'Rourke et al 2000).

O'Rourke and colleagues reported that six clinically normal sheep which were positive to an immunohistochemistry assay on third eyelid-associated lymphoid tissue developed clinical scrapie 2 to 7 months after the assay (O'Rourke et al 1998). In a later study, the same researchers reported that 27 eyelid biopsy-positive sheep progressed to confirmed scrapie within 3 to 20 months of biopsy (O'Rourke et al 2000). As has been reported for tonsil biopsies, PrP^{sc} is detectable in third eyelid biopsies around halfway through the incubation period (Ryder et al 2004).

In a large field trial in Wyoming, O'Rourke and colleagues found that suitable biopsy samples could be collected from around 80% of sheep. There was a lower percentage of suitable samples in sheep older than 3 years (O'Rourke et al 2002). A preliminary estimate of the sensitivity of the third eyelid biopsy test in preclinical scrapie was 87.8% (36 out of 41 sheep) (O'Rourke et al 2000). In a later report, the sensitivity of the third eyelid test was estimated to be around 85% to 90% (O'Rourke et al 2002).

Third eyelid testing is most useful in sheep between the ages of 14 and 36 months, as older sheep have smaller areas of lymphoid tissue and the number of lymphoid follicles decreases with age. For this reason, the third eyelid biopsy test for scrapie becomes less useful as sheep get older (O'Rourke et al 2002).

In the first report by O'Rourke and colleagues, the PrP genotype was determined for 17 sheep which showed positive immunohistochemistry staining. All were of scrapie-susceptible genotypes (O'Rourke et al 1998). It has been shown that the accumulation of PrP^{sc} in the lymphoid tissues of tonsil and nictitating membrane varies according to the genotype of the sheep. VRQ/VRQ and VRQ/ARQ genotypes, and to a lesser extent ARQ/ARQ, have a high prevalence of detectable PrP^{sc} accumulation in tonsil and nictitating membrane, but detection in the tonsil is "more reliable" than at the second site (Ryder et al 2004).Other studies have demonstrated the superiority of tonsil biopsy compared to third eyelid biopsy. Andreoletti and colleagues reported that PrP^{sc} was detected in the tonsils as early as 3 months of age but not until 5 months of age in the third eyelid (Andreoletti et al 2000) while Vascellari and co-workers reported that PrP^{sc} was detected in tonsils of 29 of 32 sheep infected preclinically with scrapie but was detected in third eyelid in only 18 of the same 32 sheep (Vascellari et al 2005).A recent study concluded that one drawback of the third eyelid test is that

a large proportion of samples contain insufficient lymphoid tissue for evaluation (Dennis et al 2009).

Biopsies on RAMALT: Preclinical diagnosis of scrapie by biopsy of third eyelid or tonsil is not applicable to large-scale testing because of technical and methodological constraints (González et al 2005, González et al 2008b). Furthermore, in cases of scrapie in sheep of less-susceptible genotypes, the distribution of PrP^{sc} is largely restricted to the gut-associated lymphatic tissue (Konold et al 2008). These limitations led González and co-workers to examine the potential of testing recto-anal mucosa-associated lymphoid tissue (RAMALT) for accumulation of PrP^{sc} (González et al 2005, González et al 2006). They demonstrated that this technique is applicable to large scale field operations (González et al 2008b) and that the efficiency of detection of PrP^{sc} during the incubation period by biopsy of RAMALT is the same as that of biopsy of tonsil (González et al 2008a, González et al 2008b).

Preclinical diagnosis of scrapie through biopsy of RAMALT has been shown in a number of studies to be useful. PrP^{sc} was consistently found in RAMALT of clinically affected confirmed cases of scrapie. Of 244 sheep, 3 of which had no accumulation of PrP^{sc} in the CNS, only seven were negative by rectal biopsy (diagnostic sensitivity 97.1%) (González et al 2006). PrP^{sc} also accumulates in RAMALT in a high proportion (104 out of 121 or 86%) of cases of preclinical scrapie in naturally infected sheep (González et al 2005, González et al 2006, González et al 2008b). In a study of experimentally inoculated sheep, PrP^{sc} was not detected in RAMALT from the single ARQ/VRQ sheep with clinical signs (Espenes et al 2006).

In experiments in which 48 sheep were experimentally inoculated with scrapie, PrP^{sc} was detected in RAMALT biopsy from susceptible genotypes (VRQ/VRQ and ARQ/VRQ) which developed clinical scrapie 7 to 12 months after inoculation (Espenes et al 2006). González and co-workers showed that PrP^{sc} can be detected in biopsies of RAMALT as early as 4 to 5 months of age in VRQ/VRQ sheep exposed naturally to scrapie but in ARQ/VRQ sheep, PrP^{sc} is not detectable until 12 months of age (González et al 2008a). PrP^{sc} accumulation in RAMALT was detected in all breeds, ages and PrP genotypes in which scrapie was confirmed (González et al 2005, González et al 2008b).

Scrapie can be diagnosed through detection of PrP^{sc} in RAMALT about halfway through the incubation period of scrapie (González et al 2005, González et al 2008a).

On the basis of the observation that the correlation between results obtained by immunohistochemistry and western blot is high, González and co-workers (2008b) developed and validated an enzyme immunoassay applicable to biopsy samples obtained from rectal mucosa (González et al 2008b). Compared to immunohistochemistry, the enzyme immunoassay developed by González and

colleagues (2008b) had a relative specificity of 99.2% and a relative sensitivity of 93.5% (González et al 2008b). When evaluated for its ability to detect PrP^{sc} in the preclinical stages of the disease, the enzyme immunoassay detected 95% of infected animals when animals were sampled once but 100% when they were sampled on two occasions (González et al 2008b). The enzyme immunoassay can be used to screen conventional sheep flocks to determine their scrapie status (González et al 2008b).

In a large trial carried out under field conditions, which compared RAMALT and third eyelid biopsies in sheep which were considered as being high risk for scrapie but which were mostly free from clinical signs, Dennis and co-workers reported that the diagnostic sensitivity of rectal biopsy was 88% (40 out of 46) and third eyelid biopsy 87.0% (117 out of 133). Only three sheep confirmed as having scrapie showed clinical signs. Biopsy of RAMALT gave false negative results in 16 of the 113 sheep confirmed as having scrapie (Dennis et al 2009).

González and colleagues caution that an individual negative result should never be considered proof that the animal is not infected and, as with the other tests used to detect PrP^{sc} in lymphoid tissue, a negative result to the enzyme immunoassay does not rule out infection. Repeated testing increases diagnostic sensitivity (González et al 2008b). In a personal communication with the risk assessor, Dr Martin Jeffrey, one of the co-authors of the papers describing the studies on RAMALT biopsies, stated "We would not maintain that RAMALT biopsy could be used as a definitive test for the presence of infectivity … It would therefore be of little use in [New Zealand] as a border control."[17]

Biopsy of RAMALT and examination for accumulation of PrP^{sc} can be used to diagnose scrapie in goats (González et al 2008a).

New tests to detect PrP^{sc}?:   New methods for the detection of PrP^{sc} are likely to be developed in the near future. For example, although currently limited to research laboratories, protein-misfolding cyclic amplification (PMCA) (Saborio, Permanne and Soto 2001) shows considerable promise as a sensitive test capable of detecting minute amounts of PrP^{sc} in the blood of sheep with scrapie (Thorne and Terry 2008).

Limitations of tests to detect PrP^{sc} in peripheral lymphoid tissues: While all of the tests based on detection of PrP^{sc} accumulation in lymphoid tissue are capable of diagnosing scrapie infection from around half-way through the incubation period, they obviously will fail to detect infection earlier than this. As several researchers have pointed out, a negative test result on lymphoid tissue is no guarantee that the sheep (or goat) is free from scrapie infection (Schreuder et al 1995, Hörnlimann et al 2007c, González et al 2008b). While immunohistochemical examination of lymphoid tissues may detect preclinical

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[17] M. Jeffrey. Personal communication with SC MacDiarmid. 14 May 2009.

cases of scrapie before PrP^{sc} is detectable in the central nervous system, some infected sheep of less-susceptible genotypes, even in advanced clinical stages of the disease, may not have PrP^{sc} detectable in lymphoid tissues (Monleon et al 2005).

Additionally, the effect of PrP genotype on the efficacy of tests on lymphoid tissue can vary between breeds and between scrapie strains (Andreoletti et al 2000). For example, PrP^{sc} was detected in the mesenteric lymph nodes of sheep of the highly susceptible VRQ/VRQ genotype (see Table 2) as early as 2 to 9 months of age while in clinically affected VRQ/ARR sheep, PrP^{sc} accumulation was detected only in CNS and not in lymphoid tissues (Andreoletti et al 2000). This latter finding led Andreoletti and co-workers to propose that sheep carrying the ARR allele do not accumulate PrP^{sc} in lymphoid tissue (Andreoletti et al 2000). However, this suggestion has been shown to be incorrect by the findings of González and colleagues (2006) who detected PrP^{sc} accumulation in RAMALT of 8 of 10 infected sheep of the VRQ/ARR genotype.

Studies on the detection of PrP^{sc} in peripheral lymphoid tissues have generally been performed on sheep of the most common *Prnp* genotypes. The effect of minor polymorphisms (not associated with resistance) on peripheral lymphoid tissue ante-mortem testing is not well described. Laegreid et al (2008) showed prolonged incubation periods in ARQ/ARQ sheep with the M/T mutation at codon 112. Ongoing studies at USDA Pullman[18] suggest a concomitant delay in the appearance of detectable PrP^{sc} in lymphoid tissues in sheep of this genotype. Likewise, sheep with the L/F polymorphism at codon 141 showed a prolonged incubation period with delayed appearance of sparse accumulations of PrP^{sc} in any lymphoid tissue until close to the onset of clinical disease.

Biopsy of RAMALT for the detection of PrP^{sc} in lymphoid tissues is not useful for diagnosis of so-called **atypical scrapie**, because in that condition there is no involvement of the lymphoreticular system (González et al 2008b) and it is doubtful that there is any aetiologic, pathologic or epidemiologic relationship between atypical scrapie and 'classical' scrapie (see Appendix 4).

Likelihood of identifying a flock as infected using biopsy of lymphoid tissue: As stated above, the sensitivity of tests which examine lymphoid tissues for accumulations of PrP^{sc} is not sufficient to give good assurances on the scrapie-free status of an individual sheep or goat.

While in northern hemisphere flocks infected with scrapie, annual mortalities are said to typically range from 3 to 5% (Smit et al 2002, Hörnlimann et al 2007c), surveys of various designs have mostly estimated within-flock prevalences in Great Britain and the Netherlands to be lower than these figures, with prevalences of 0.32, 0.37, 0.8-1.2, 1.27 and 6.6 per 100 sheep per annum being

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[18] K O'Rourke, personal communication with SC MacDiarmid, 8 July 2009.

suggested (Sivam et al 2006, Hoinville et al 2000, Gubbins 2005, Schreuder at al 1993, Tongue et al 2005). The best estimate for Great Britain is probably that of Ortiz-Pelaez and Del Rio Vilas (2009) in which, on the basis of culling as part of a national scrapie control programme, they demonstrated the mean within-flock prevalence in infected flocks to be 0.65% per annum (95% confidence intervals 0.55-0.75%).

Given that the diagnostic sensitivity of the RAMALT biopsy test seems to be around 86 to 87% (González et al 2005, González et al 2006, González et al 2008b, Dennis 2009), this test could be applied on a flock basis to give a reasonable confidence of identifying flocks infected with scrapie.

Table 5: **Probability of failing to identify a scrapie-infected flock using whole-of-flock biopsy of RAMALT. Sensitivity of test 86%.**

Number of infected sheep in the flock	Probability of failing to identify flock as infected	Probability of identifying flock as infected
1	0.14	0.86
2	0.0196	0.98
3	0.002744	0.997256

It can be seen from Table 5 that a whole-of-flock test on a biopsy of RAMALT would have a high probability of identifying as infected a flock which had as few as three infected animals at least half-way through the incubation period of scrapie. However, the results of the survey by Ortiz-Pelaez and Del Rio Vilas (2009) suggest that relatively few infected flocks are likely to have more than one detectable infected sheep at any one time.

Of course, the British and Dutch findings may not be universally applicable. In Iceland, an examination of sheep in flocks completely culled for scrapie has revealed a relatively high incidence of subclinical/preclinical infection (Georgsson et al 2008). That is, in that country, in an infected flock, there is a relatively high likelihood that there would be more than one infected animal at any one time.

3.3.4. The safety of semen

There are good grounds for considering that semen is not a vehicle for scrapie (see Section 3.1.4.5). Imports of semen, with no additional safeguards, would be unlikely to introduce scrapie along with new bloodlines of sheep and goats.

3.3.5. The safety of embryo transfers

There is very strong evidence that embryos, collected according to the recommendations of the OIE, are not a vehicle for scrapie (see Section 3.1.4.5).

Imports of embryos, without additional safeguards, would be unlikely to introduce scrapie along with new bloodlines of sheep and goats.

While science cannot prove that any event **cannot** occur, the more trials that are completed without the event occurring, the more confident one can be that it will not occur in the next trial. As discussed earlier (see Section 3.1.4.5), three methodologically-sound studies have been carried out to determine whether scrapie can be transmitted by embryos collected from naturally-infected sheep (Wang et al 2001, Wang et al 2002, Foster et al 2006b, Low et al 2009).Combining the results of these studies gives us 95 offspring of susceptible genotypes that survived long enough to demonstrate that transmission of scrapie had not occurred. The OIE's *Handbook on Import Risk Analysis for Animals and Animal Products* (OIE 2004) describes the use of the beta distribution to calculate the likelihood of the unwanted event (scrapie transmission) occurring in the next trial (that is, the transfer of an embryo from an infected donor). In this instance, where there has been no transmission in 95 trials, the likelihood of it occurring for the first time in the 96th trial (assuming it is biologically feasible) is not greater than 0.01 (1%). In other words, having demonstrated that transmission has not occurred in 95 embryo transfers, we can expect that it will not occur on the 96th transfer either.

3.3.6. Selection of donors on basis of age

One measure by which the risk of introducing scrapie could be reduced would be to require that all donors be over the age at which scrapie commonly manifests. That is, if an animal were older than a given age and were still clinically healthy, it could be considered that there was little likelihood of it carrying scrapie. For example, New Zealand's SFAPs are based on the assumption that 76% of infected sheep will manifest signs of clinical scrapie by 5 years of age (MacDiarmid 1996).

Greater assurance might be gained if animals of only certain genotypes were eligible for inclusion in an importation programme. For example, in the study by Foster and colleagues (2006b), the mean age of natural scrapie cases in the highly-susceptible VRQ/VRQ genotype was 48 months (Foster et al 2006b). However, the usefulness of restricting eligible animals to highly susceptible genotypes (Table 2) is limited because national scrapie control programmes are significantly reducing the incidence of such genotypes in national flocks (Table 4).

3.3.7. Quarantine of offspring

The purpose of quarantine in an importation programme would be to hold the embryo-derived or semen-derived animals for a period sufficiently long that the disease would have time to manifest itself, should it be present. The problem with using quarantine as a measure to reduce the risk of introducing scrapie is the

very long periods necessary to give reasonable assurances of freedom. For example, as reported above, the majority of cases in sheep manifest between 2 and 5 years of age (Hoinville 1996).A large study in the UK demonstrated that 80% of scrapie cases died aged 2 to 4 years (Baylis et al 2004). New Zealand's SFAPs were based on the assumption that 76% of infected sheep will manifest signs of clinical scrapie by 5 years of age (MacDiarmid 1996). It has long been considered that such quarantine is, in the absence of other safeguards, insufficient to insure that scrapie is not introduced into New Zealand with importations of sheep or goats.

3.3.8. Options for embryos

The benchmark against which different options or combinations of options must be assessed is the safety provided by the SFAPs under which the previous importations of sheep (Table 1) have been made. The SFAPs in place since 1991 involved an embryo transfer barrier between the foreign donor sheep and the New Zealand national flock, a bioassay into sentinel goats of mesenteric lymph node biopsies from the donor sheep, and a 3½ to 5 year quarantine of the embryo derived offspring (MacDiarmid 1996). MAF risk assessments, reviewed and endorsed by the government's independent BSE Expert Science Panel, concluded that the SFAPs provide firm guarantees against the introduction of either scrapie or BSE (MAF Biosecurity New Zealand 2008b)..

3.3.8.1. The international standard

The *Code* (Appendix 3) recommends the following;

Article 14.9.9.

Recommendations for importation from countries or zones not considered free from scrapie

for embryos/oocytes of sheep and goats

Veterinary Authorities should require the presentation of an *international veterinary certificate* attesting that:

1. in the country or *zone*:

    a) the *disease* is compulsorily notifiable;

    b) an awareness, *surveillance* and monitoring system as referred to in Article 14.9.2. is in place;

    c) affected sheep and goats are slaughtered and completely destroyed;

    d) the feeding to sheep and goats of *meat-and-bone meal* or *greaves* of ruminant origin has been banned and effectively enforced in the whole country;

2. the donor animals either have been kept since birth in a free *establishment*, or meet the following conditions:

a) are permanently identified, to enable trace back to their *establishment* of origin;

b) have been kept since birth in *establishments* in which no *case* of scrapie had been confirmed during their residency;

c) showed no clinical sign of scrapie at the time of embryo/oocyte collection;

3. the embryos/oocytes were collected, processed and stored in conformity with the provisions of Chapter 4.7.

Since the first importations of sheep embryos into New Zealand in the 1980s, a substantial body of evidence has accumulated to show that embryos with intact *zona pellucida* washed according to the procedures of the International Embryo Transfer Society are highly unlikely to transmit scrapie. The international standard for importing embryos from a country not considered free from scrapie provides very strong protection against the introduction of the disease.

3.3.8.2. Importation of embryos from scrapie-free countries

Very few countries are generally accepted as free from scrapie. Apart from New Zealand, the only countries widely considered as scrapie-free are Australia, South Africa and Argentina. Requiring that embryos be sourced only from countries free from scrapie would probably provide very little assurance above that provided by the international standard outlined above and would prevent New Zealand importers securing access to certain bloodlines or breeds of particular merit.

3.3.8.3. Importation of embryos from scrapie-free flocks

The *Code* (Appendix 3) provides rigorous criteria by which a flock (or 'establishment' in the language of the *Code*) may be considered free from scrapie. However, the recommendations for embryos in the *Code* do not restrict embryos to those meeting the *Code*'s own criteria for flock freedom from scrapie. Requiring that embryos be sourced only from flocks free from scrapie would provide little in additional biosecurity.

3.3.8.4. Restrict donors to particular genotypes

It is extremely unlikely that sheep of the ARR/ARR genotype would be infected by scrapie (see Section 3.1.4.4 and particularly Table 2). Sheep of the genotypes ARR/AHQ, ARR/ARH and ARR/ARQ are also genetically resistant to scrapie. Sheep selected as embryo donors could be restricted to these genotypes (that is NSP categories 1 and 2, Table 3), making it improbable that they would be pre-clinically infected with scrapie. Such a requirement might provide additional security but could limit access to certain breeds in which these genotypes are uncommon.

Rather than restrict embryo donors to certain genotypes, it would be less limiting if ewes of any genotype were permitted, but only rams of the ARR/ARR genotype be used to inseminate them. This would insure that all embryos carried at least one ARR allele and thus be resistant to infection with scrapie. Restriction of genotype could be used in combination with other measures, possibly achieving greater reduction of risk.

Restriction of genotype cannot, at this stage, be considered useful for managing the scrapie risk in importation of goat germplasm. The genetic control of scrapie in goats is not sufficiently well understood for recommendations to be made (see Section 3.1.4.4).

3.3.8.5. Restrict donors to animals over a certain age

It could be argued that one could restrict embryo donors to ewes over a certain age, especially if the ewes were of susceptible genotypes. However, there is little additional security to be gained from such restrictions. Although scrapie infection is acquired mainly during the immediate post-parturient period, horizontal spread between adults does occur and infection can be acquired later in life.

3.3.8.6. Restrict donors to animals negative on RAMALT biopsy

The sensitivity of all the tests based on detection of PrP^{sc} in accessible lymphoid tissue is not sufficient to provide good assurances about the scrapie-freedom of individual sheep and goats. However, they could be used to determine flock status (see Table 5). Biopsy of RAMALT is superior to that on tonsil or third eyelid for technical and methodological reasons. Testing the whole flock by biopsy of RAMALT could provide reasonable assurance that the flock was scrapie free, so long as the flock was a closed one, with no introductions for several years. Whole of flock test by RAMALT biopsy would provide little security if the flock had been assembled recently for the purpose of an embryo collection programme.

There would be little point in conducting RAMALT biopsies on sheep of resistant genotypes as such animals will not have accumulations of PrP^{sc} in lymphoid tissue.

3.3.8.7. Restrict donors to animals negative on bioassay

New Zealand's SFAPs in the 1990s relied on bioassay of mesenteric lymph node material carried out in sentinel goats. Such bioassay is probably a sensitive tool for detecting infection in donor sheep (see Section 3.3.3.1). However, such bioassays are expensive; the sentinel goats were kept in quarantine for 3 years before they were considered to be uninfected. There were also animal welfare issues surrounding the intracerebral inoculation of material into goats. With the greater understanding of scrapie that has been acquired since the 1990s, and with the development of tests for the detection of PrP^{sc} in lymphoid tissues,

bioassays may no longer be considered acceptable. Whole of flock screening of donors by RAMALT would provide almost as much security as testing the individual donors by bioassay.

3.3.8.8.   Quarantine of offspring

Prolonged quarantine of the offspring resulting from the implantation of imported embryos has been a significant component of the SFAPs which have operated since the 1990s. However, with the greater understanding of scrapie that has been acquired since the 1990s, especially with the much better evidence for the safety of embryo transfer, it is probable that prolonged quarantine would add little in additional biosecurity but definitely adds very major cost. Although assurances about the scrapie-free status of the donor flock (such as those provided by the *Code*'s Article defining flock freedom, see Appendix 3) are probably unnecessary, given the evidence for the safety of embryo transfer, they would provide greater assurance than prolonged quarantine of embryo-derived offspring and at lower cost to the importer. Requiring that donor flocks meet the criteria for flock freedom would effectively move the risk management off shore.

3.3.9. Options for semen

3.3.9.1.   The international standard

There is good evidence that semen is unlikely to transmit scrapie. The recommendations in *Code* (Appendix 3) for importing semen from a country not considered free from scrapie do not restrict importations to flocks meeting the OIE's criteria for flock freedom.

Article 14.9.8.

Recommendations for importation from countries or zones not considered free from scrapie

for semen of sheep and goats

Veterinary Authorities should require the presentation of an *international veterinary certificate* attesting that:

1.   the donor animals:

  a)   are permanently identified, to enable trace back to their *establishment* of origin;

  b)   have been kept since birth in *establishments* in which no *case* of scrapie had been confirmed during their residency;

  c)   showed no clinical sign of scrapie at the time of semen collection;

2.   the semen was collected, processed and stored in conformity with the provisions of

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The international standard for importing sheep and goat semen from a country not considered free from scrapie provides strong protection against the introduction of the disease. Because fewer experiments have been conducted to examine the safety of semen compared to embryos, measures additional to those in the international standard might be considered warranted.

3.3.9.2. Importation of semen from scrapie-free countries

Very few countries are generally accepted as free from scrapie. Apart from New Zealand, the only countries widely considered as scrapie-free are Australia, South Africa and Argentina. Requiring that semen be sourced only in countries free from scrapie could provide some additional safety but would prevent New Zealand importers gaining access to certain bloodlines or breeds of particular merit.

3.3.9.3. Importation of semen from scrapie-free flocks

The OIE's *Code* (Appendix 3) provides rigorous criteria by which a flock (or 'establishment' in the language of the *Code*) may be considered free from scrapie. However, the recommendations for semen in the *Code* do not restrict imports to flocks meeting the criteria for freedom from scrapie. Requiring that semen be sourced only from flocks free from scrapie would provide additional biosecurity if such were considered necessary.

3.3.9.4. Restrict rams to particular genotypes

It is extremely unlikely that rams of the ARR/ARR genotype would be infected by scrapie (see Section 3.1.4.4 and particularly Table 2). Sheep of the genotypes ARR/AHQ, ARR/ARH and ARR/ARQ are also genetically resistant to scrapie. Rams selected as semen donors could be restricted to these genotypes (that is NSP categories 1 and 2, Table 3), making it improbable that they would be pre-clinically infected with scrapie. Restriction of genotype could be used in combination with other measures to achieve greater reduction of risk.

Restriction of genotype cannot, at this stage, be considered useful for managing the scrapie risk in importation of goat semen. The genetic control of scrapie in goats is not sufficiently well understood for recommendations to be made (see Section 3.1.4.4).

3.3.9.5. Restrict rams to animals over a certain age

There would be little to be gained from restricting semen donors to rams over a certain age. Although scrapie infection is acquired mainly during the immediate

post-parturient period, horizontal spread between adults does occur and infection can be acquired later in life.

3.3.9.6. Restrict donors to rams negative on RAMALT biopsy

The sensitivity of all the tests based on detection of PrP^{sc} in accessible lymphoid tissue is insufficient to provide good assurances about the scrapie-freedom of individual sheep and goats.

3.3.9.7. Restrict donors to rams negative on bioassay

While bioassays in sentinel goats are probably a sensitive tool for detecting infection in donor sheep, such bioassays are expensive; in the SFAPs of the 1990s, the sentinel goats were kept in quarantine for 3 years before they were considered to be uninfected. There were also animal welfare issues surrounding the intracerebral inoculation of material into goats. With the greater understanding of scrapie that has been acquired since the 1990s, bioassays may no longer be considered acceptable.

3.3.9.8. Quarantine of offspring

Prolonged quarantine of the offspring resulting from imported semen could be considered as a useful measure, analogous to the prolonged quarantine of the SFAPs that have been operated since the 1990s. However, with the greater understanding of scrapie that has been acquired since the 1990s, especially with the much better evidence for the safety of semen, it is probable that prolonged quarantine would add little in additional biosecurity but definitely adds very major cost. Although assurances about the scrapie-free status of the donor flock (such as those provided by the *Code*'s Article defining flock freedom, see Appendix 3) are probably unnecessary, given the evidence for the safety of semen, they would provide greater assurance than prolonged quarantine of embryo-derived offspring and at lower cost to the importer. Requiring that donor flocks meet the criteria for flock freedom would effectively move the risk management off shore.

Restricting semen donors to rams of the ARR/ARR genotype would provide good additional safety at little additional cost. However, as pointed out above, there is insufficient information at this stage to make recommendations regarding the selection of goats to those of particular genotypes.

APPENDIX 1

'Atypical' scrapie

Background

So-called 'atypical scrapie' is a rare condition that has been detected through the intense surveillance programmes undertaken in the European Union following concerns that BSE might have entered European sheep flocks through the use of rations containing meat-and-bone meal. An examination of the epidemiology of atypical scrapie suggests that the condition has been present, and widely distributed, in Europe for a long time (Bruce et al 2007, Foster et al 2008). The fact that the majority of 'cases' have been found in clinically normal animals (Benestad et al 2008) partly explains why this condition was not detected earlier; it causes no significant wastage amongst livestock and hence its presence would not have been noticed. "The widespread distribution of 'atypical' scrapie cases in Europe suggests that they represent a previously unrecognised isolate or isolates rather than one that has recently dispersed between countries." (EFSA 2005).

Atypical scrapie is unrelated to classical scrapie

Atypical scrapie is not related to classical scrapie. It is a distinct condition; clinically, epidemiologically, histopathologically and biochemically (Benestad et al 2008, Foster et al 2008). For example, EFSA (2005) concluded "An operational definition of atypical scrapie in small ruminants is possible ... This definition is provided in juxtaposition with similar definitions for scrapie and BSE in small ruminants." Similarly, the British SEAC Sheep Subgroup (SEAC 2006) concluded "…that, on the basis of a number of characteristics, atypical scrapie could reliably be distinguished both from classical scrapie and from experimental BSE in sheep." The same SEAC subgroup considered atypical scrapie to be "… a subset of TSE cases in sheep which are more closely related to each other and distinct from classical scrapie…" and "…it may be more appropriate to consider atypical scrapie as a distinct TSE of small ruminants, and not simply a variant of what is now called classical scrapie." They went on to state "There is no evidence for a direct link between the occurrence of classical scrapie and atypical scrapie, consistent with the view that classical scrapie and atypical scrapie should be considered as independent TSEs."

An OIE *ad hoc* group convened to consider the implications of atypical scrapie concluded that "There is currently no epidemiological evidence of an association between classical and atypical scrapie." (OIE 2007).

Atypical scrapie tends to occur in genotypes that are associated with resistance to infection with classical scrapie (Hunter 2007, Fediaevsky et al 2008, Luhken et al 2007, Benestad et al 2008). Sheep with genotypes most susceptible to classical scrapie appear unaffected by atypical scrapie. Atypical scrapie is often linked to the ARR and AHQ alleles which tend to be associated with moderate to marked resistance to classical scrapie. Atypical scrapie cases frequently have phenylalanine (F) rather than leucine (L) at codon 141 (Simmons et al 2009).

Atypical scrapie is a rare condition

Active surveillance has found atypical scrapie in most European countries, the Falkland Islands and North America. Cases appear to be evenly distributed and there has seldom been more than a single case detected in any one flock (Benestad et al 2008). Atypical scrapie is not a cause of significant livestock wastage; it is a rare condition, as clearly demonstrated by the results of the extensive surveillance programmes undertaken in the European Union. For example, in the United Kingdom in 2006, out of 87,912 sheep samples from five different surveillance streams, 223 were diagnosed as classical scrapie and 60 as atypical scrapie cases. That is, a total of 0.08% tested positive for atypical scrapie (Del Rio Vilas et al 2007). European Union-wide surveillance in 2006 involved testing 1,035,065 sheep for scrapie (European Commission 2007). That surveillance detected scrapie in 3,507 sheep (0.34%) and only 365 of these (0.03%) were atypical scrapie.

In other studies (Luhken et al 2007) it has been noted that "… only a single scrapie positive sheep was found in more than 90% of the flocks where atypical scrapie was diagnosed…" and "One of the most striking aspects of atypical scrapie is that only a single scrapie-positive sheep per affected flock was identified in most cases."

The very low incidence of atypical scrapie means that it cannot be considered a significant source of livestock wastage. Indeed, the evidence from various studies suggests that the age-at-onset of clinical signs for atypical scrapie is much later than the commercial life-span of a sheep (McIntyre at al 2008).

Atypical scrapie is probably not contagious, possibly spontaneous

PrP^{sc} is not detectable in the lymphoreticular system of atypical scrapie cases (Benestad et al 2008) and it appears that sheep affected by atypical scrapie may not excrete the agent and atypical scrapie may not even be transmissible naturally between sheep (EFSA 2003, de Bosschere et al 2007, Jeffrey and González 2007, Simmons et al 2007, Green et al 2007, Fediaevsky 2008, Simmons et al 2009). A case control study of atypical scrapie cases in France found no evidence that the condition is contagious (Fediaevsky at al 2009). Experimental transmission of atypical scrapie has not been achieved by the oral route (Simmons et al 2009). Experimentally, it has been transmitted by

intracerebral inoculation but this is no indication that it is contagious. In fact, there are a number of researchers (Benestad et al 2003, de Bosschere et al 2007, Nentwig et al 2007, McIntyre et al 2008, Benestad et al 2008, Foster et al 2008, Simmons et al 2009, Fediaevsky et al 2009) who have speculated that atypical scrapie may arise "spontaneously", in the same way that sporadic CJD of humans occurs. For example, "…atypical scrapie may be a spontaneous disease like sporadic Creutzfeldt-Jakob disease (CJD) in humans…" (Luhken et al 2007) and "…One hypothesis is that this represents a spontaneous genetic disease in sheep similar to familial forms of TSE in man …" (Nentwig et al 2007).

APPENDIX 2

BSE in sheep and goats

Sheep and goats can be experimentally infected with BSE, resulting in a disease the clinical signs of which are indistinguishable from classical scrapie, albeit with a shorter clinical course (Foster et al 1999, OIE 2002, Schreuder and Somerville 2003, Dustan et al 2008). Sheep and goat populations in Europe were exposed to the same meat-and-bone meal concentrate rations responsible for spreading BSE (OIE 2002, Schreuder and Somerville 2003, Stack et al 2006). Writing in 2002, Schreuder and Somerville assessed the risk that BSE could be present in European sheep as "hypothetical" (Schreuder and Somerville 2003). In 2002 an OIE expert group concluded that "it is at least theoretically possible that BSE cases could have occurred in sheep and goats in the field, primarily in countries where BSE risk has been identified" (OIE 2002).

Schreuder and Somerville pointed out that once BSE were to be introduced into a sheep population, it would likely be recycled and amplified by horizontal and vertical transmission, in the manner of scrapie (Schreuder and Somerville 2003).

However, despite these concerns, intensive surveillance has not detected BSE in European sheep flocks and only two cases have been found amongst the tens of thousands of goats examined for the presence of the disease. Stack and colleagues examined 2,147 samples from sheep diagnosed with scrapie between 1998 and 2004 without finding any evidence of BSE infection in British flocks (Stack et al 2006). The extensive surveillance carried out in the UK up to the end of November 2005 showed that BSE, if it had ever entered the UK sheep flocks, could not be accounting for more than 0.54% of all sheep 'scrapie' cases (SEAC 2006). The British Spongiform Encephalopathy Advisory Committee (SEAC) concluded in 2007 that "… the most likely prevalence of BSE in UK sheep is zero or at most extremely low" (SEAC 2007). In December 2006 SEAC concluded that the risk of the UK national flock being infected with BSE is negligible (Dawson and Del Rio Vilas 2008). The prevalence of BSE in the British national flock is close to zero, if it is present at all (Konold et al 2008).

The situation is the same in the European Union as a whole. In 2007, the European Food Safety Authority concluded that the most likely prevalence of BSE in European sheep was zero but that in a "high risk sub-group" of European countries it could be as high as 0.3 to 0.5 cases per 10,000 sheep at slaughter (EFSA 2007).

Two cases of BSE in goats have been reported, one in France and the other in the UK (Dustan et al 2008). However, no other cases have been detected,

despite 611,151 goats being tested in the years 2002 to 2006 inclusive (European Commission 2007).

Even if this intensive surveillance had revealed the presence of BSE in European sheep and goats populations, the likelihood of importing BSE in germplasm would be negligible. Studies in which embryos have been collected from experimentally-infected goats demonstrated that BSE was not transmitted to embryo-derived offspring (Foster et al 1999). Neither was BSE transmitted to recipients of embryos collected from experimentally infected goats or to kids born naturally to goats experimentally infected with BSE (Foster et al 1999).

In early 1996, at a time when MAF was operating a SFAP for sheep germplasm imported from the UK, there was concern that there might be a BSE risk associated with the importation. However, the issue was considered by the government's independent BSE Expert Science Panel which first met on 27 March 1996 at the Ministry of Research, Science and Technology. In a report dated 18 April 1996 the Panel concluded; "That the controls imposed by MAF on the importation of ovine material from the United Kingdom to exclude scrapie, would also be adequate to prevent the introduction of BSE to New Zealand." (BSE Expert Science Panel 1996).

This conclusion is reported on the MAF web site where it is stated that; "MAF risk assessments, reviewed and endorsed by the government's independent BSE Expert Science Panel, concluded that the Scrapie Freedom Assurance Programmes (SFAPs), under which sheep have been imported to New Zealand since the mid-1980s, provide firm guarantees against the introduction of either of the TSEs…" (MAF Biosecurity New Zealand 2008b).

APPENDIX 3

Terrestrial animal health code chapter

CHAPTER 14.9.

SCRAPIE

Article 14.9.1.

General provisions

Scrapie is a neurodegenerative *disease* of sheep and goats. The main mode of transmission is from mother to offspring immediately after birth and to other susceptible neonates exposed to the birth fluids and tissues of an infected animal. Transmission occurs at a much lower frequency to adults exposed to the birth fluids and tissues of an infected animal. A variation in genetic susceptibility of sheep has been recognised. The *incubation period* of the *disease* is variable; however, it is usually measured in years. The duration in *incubation period* can be influenced by a number of factors including host genetics and strain of agent.

Scrapie is not considered to pose a risk to human health. The recommendations in this Chapter are intended to manage the animal health risks associated with the presence of the scrapie agent in sheep and goats. The Chapter does not cover so-called 'atypical' scrapie which is clinically, pathologically, biochemically and epidemiologically unrelated to 'classical' scrapie, may not be contagious-and may, in fact, be a spontaneous degenerative condition of older sheep.

1. When authorising import or transit of the following *commodities* derived from sheep or goats and any products made from these *commodities* and containing no other tissues from sheep or goats derived, *Veterinary Authorities* should not require any scrapie-related conditions, regardless of the scrapie risk status of the sheep and goat populations of the *exporting country*, *zone* or *compartment*:

    a) *meat* (excluding materials as referred to in Article 14.9.11.);

    b) hides and skins;

    c) gelatine;

    d) collagen prepared from hides or skins;

    e) tallow (maximum level of insoluble impurities of 0.15% in weight) and derivatives made from this tallow;

    f) dicalcium phosphate (with no trace of protein or fat);

    g) wool or fibre.

2. When authorising import or transit of other *commodities* listed in this Chapter, *Veterinary Authorities* should require the conditions prescribed in this Chapter relevant to the scrapie risk status of the sheep and goat populations of the *exporting country*, *zone* or *compartment*.

Standards for diagnostic tests are described in the *Terrestrial Manual*.

Article 14.9.2.

Determination of the scrapie status of the sheep and goat populations of a country, zone, compartment or establishment

The scrapie status of the sheep and goat populations of a country, *zone, compartment* or-establishment should be determined on the basis of the following criteria:

1. the outcome of a *risk assessment* identifying all potential factors for scrapie occurrence and their historic perspective, in particular the:

    a) importation or introduction of sheep and goats or their semen, embryos/oocytes potentially infected with scrapie;

    b) extent of knowledge of the population structure and husbandry practices of sheep and goats;

    c) feeding practices, including consumption of *meat-and-bone meal* or *greaves* derived from ruminants;

    d) importation of milk and milk products of sheep or goats origin intended for use in feeding of sheep and goats.

2. an on-going awareness programme for veterinarians, farmers, and workers involved in transportation, marketing and *slaughter* of sheep and goats to facilitate recognition and encourage reporting of all animals with clinical signs compatible with scrapie;

3. a *surveillance* and monitoring system including the following:

    a) official veterinary *surveillance*, reporting and regulatory control in accordance with the provisions of Chapter 1.4.;

    b) a *Veterinary Authority* with current knowledge of, and authority over, all *establishments* which contain sheep and goats in the whole country;

    c) compulsory notification and clinical investigation of ~~all~~ sheep and goats showing clinical signs compatible with scrapie;

    d) examination, in accordance with the *Terrestrial Manual*, in a *laboratory* of appropriate material from sheep and goats older than 18 months displaying clinical signs compatible with scrapie;

    e) maintenance of records including the number and results of all investigations for at least 7 years.

Scrapie free country or zone

Countries or *zones* may be considered free from scrapie if within the said territory:

1. a *risk assessment*, as described in point 1 of Article 14.9.2., has been conducted, and it has been demonstrated that appropriate measures are currently in place and have been taken for the relevant period of time to manage any *risk* identified and points 2 and 3 have been complied with for the preceding 7 years;

AND

2. one of the following conditions should be met:

    a) the country or the *zone* have demonstrated historical freedom taking into account the recommendations in Articles 14.9.13. and 14.9.14.(under study); or

    b) for at least 7 years, a sufficient number of representative mature culled sheep and goats over 18 months of age have been tested annually, to provide a 95% level of confidence of detecting scrapie if it is present at a prevalence rate exceeding 0.1% out of the total number of all chronic wasting conditions in the population of sheep and goats older than 18 months of age and no *case* of scrapie has been reported during this period; it is assumed that the occurrence rate of chronic wasting conditions within the population of sheep and goats older than 18 months of age is at least 1% (under study); or,

    c) all *establishments* containing sheep or goats have been accredited free as described in Article 14.9.4 bis.;

AND

3. the feeding to sheep and goats of *meat-and-bone meal* or *greaves* of ruminant origin has been banned and effectively enforced in the whole country for at least 7 years;

AND

4. introductions of sheep and goats, semen and embryos/oocytes from countries or *zones* not free from scrapie are carried out in accordance with Articles 14.9.6., 14.9.7., 14.9.8. or 14.9.9., as relevant.

Scrapie free compartment

One or more *establishments* may be considered eligible for accreditation as a scrapie free *compartment* if:

1. in the country or *zone* where the *establishments* are situated, the following conditions are fulfilled:

    a) the *disease* is compulsorily notifiable;

    b) an awareness, *surveillance* and monitoring system as referred to in Article 14.9.2. is in place;

    c) affected sheep and goats are slaughtered and completely destroyed;

d)   the feeding to sheep and goats of *meat-and-bone meal* or *greaves* of ruminant origin has been banned and effectively enforced in the whole country;

e)   an official accreditation scheme is in operation under the supervision of the *Veterinary Authority*, including the measures described in point 2 below;

2.   in the *establishments* the following conditions have been complied with for at least 7 years:

a)   sheep and goats are permanently identified and records maintained, to enable trace back to their *establishment* of birth;

b)   records of movements of sheep and goats in and out of the *establishment* are maintained;

c)   introductions of sheep and goats are allowed only from free *establishments* of an equal or higher stage in the process of accreditation; however, rams and bucks complying with the provisions in point 2 of Article 14.9.8. may also be introduced;

d)   an *Official Veterinarian* inspects sheep and goats in the *establishments* and audits the records at least once a year;

e)   no *case* of scrapie has been reported;

f)   sheep and goats of the *establishments* should have no direct or indirect contact, including shared grazing, with sheep or goats from *establishments* of a lower status;

g)   all culled sheep and goats over 18 months of age are inspected by an *Official Veterinarian*, and a proportion of those exhibiting wasting signs and all those exhibiting neurological signs are tested in a *laboratory* for scrapie. The selection of the sheep and goats to be tested should be made by the *Official Veterinarian*. Sheep and goats over 18 months of age that have died or have been killed for reasons other than routine *slaughter* should also be tested (including 'fallen' stock and those sent for emergency *slaughter*).

Article 14.9.4.bis

Scrapie free establishment

An *establishment* may be considered eligible for accreditation as a scrapie free *compartment* if:

1.   in the country or *zone* where the *establishment* is situated, the following conditions are fulfilled:

a)   the *disease* is compulsorily notifiable;

b)   an awareness, *surveillance* and monitoring system as referred to in Article 14.9.2. is in place;

c)   affected sheep and goats are slaughtered and completely destroyed;

d)   the feeding to sheep and goats of *meat-and-bone meal* or *greaves* of ruminant origin has been banned and effectively enforced in the whole country;

e)   an official accreditation scheme is in operation under the supervision of the *Veterinary Authority*, including the measures described in point 2 below;

2. in the *establishment* the following conditions have been complied with for at least 7 years:

   a) sheep and goats are permanently identified and records maintained, to enable trace back to their *establishment* of birth;

   b) records of movements of sheep and goats in and out of the *establishment* are maintained;

   c) introductions of sheep and goats are allowed only from free *establishments*;

   d) an *Official Veterinarian* inspects sheep and goats in the *establishment* and audits the records at least once a year;

   e) no *case* of scrapie has been reported;

   f) sheep and goats of the *establishment* should have no direct or indirect contact, including shared grazing, with sheep or goats from *establishments* of a lower status;

   g) all culled sheep and goats over 18 months of age are inspected by an *Official Veterinarian*, and a proportion of those exhibiting wasting signs and all those exhibiting neurological signs are tested in a *laboratory* for scrapie. The selection of the sheep and goats to be tested should be made by the *Official Veterinarian*. Sheep and goats over 18 months of age that have died or have been killed for reasons other than routine *slaughter* should also be tested (including 'fallen' stock and those sent for emergency *slaughter*).

Article 14.9.6.

Recommendations for importation from countries or zones not considered free from scrapie

for sheep and goats for breeding or rearing

Veterinary Authorities should require the presentation of an *international veterinary certificate* attesting that the animals come from an *establishment* free from scrapie as described in Article 14.9.4. bis.

OR

In cases where the animals do not come from an *establishment* free from scrapie as described in Article 14.9.4.bis , the *importing country* may require the placing of the animals in a *quarantine station* located on its territory, in conformity with the conditions stipulated in its animal health legislation.

Article 14.9.7.

Recommendations for importation from countries or zones not considered free from scrapie

for sheep and goats for slaughter

Veterinary Authorities should require the presentation of an *international veterinary certificate* attesting that:

1. in the country or *zone*:

   a) the *disease* is compulsorily notifiable;

b) an awareness, *surveillance* and monitoring system as referred to in Article 14.9.2. is in place;

c) affected sheep and goats are slaughtered and completely destroyed;

2. the sheep and goats selected for export showed no clinical sign of scrapie on the day of shipment.

Article 14.9.8.

Recommendations for importation from countries or zones not considered free from scrapie

for semen of sheep and goats

Veterinary Authorities should require the presentation of an *international veterinary certificate* attesting that:

1. the donor animals:

a) are permanently identified, to enable trace back to their *establishment* of origin;

b) have been kept since birth in *establishments* in which no *case* of scrapie had been confirmed during their residency;

c) showed no clinical sign of scrapie at the time of semen collection;

2. the semen was collected, processed and stored in conformity with the provisions of Chapter 4.5.

Article 14.9.9.

Recommendations for importation from countries or zones not considered free from scrapie

for embryos/oocytes of sheep and goats

Veterinary Authorities should require the presentation of an *international veterinary certificate* attesting that:

1. in the country or *zone*:

a) the *disease* is compulsorily notifiable;

b) an awareness, *surveillance* and monitoring system as referred to in Article 14.9.2. is in place;

c) affected sheep and goats are slaughtered and completely destroyed;

d) the feeding to sheep and goats of *meat-and-bone meal* or *greaves* of ruminant origin has been banned and effectively enforced in the whole country;

2. the donor animals either have been kept since birth in a free *establishment*, or meet the following conditions:

a) are permanently identified, to enable trace back to their *establishment* of origin;

b) have been kept since birth in *establishments* in which no *case* of scrapie had been confirmed

during their residency;

    c)   showed no clinical sign of scrapie at the time of embryo/oocyte collection;

3.   the embryos/oocytes were collected, processed and stored in conformity with the provisions of Chapter 4.7.

<center>Article 14.9.9.bis</center>

Recommendations for importation from countries or zones not considered free from scrapie

for milk and milk products of sheep or goat origin intended for use in feeding of sheep and goats

Veterinary Authorities should require the presentation of an *international veterinary certificate* attesting that the milk and milk products come from scrapie free *establishments*.

<center>Article 14.9.10.</center>

Recommendations on meat-and-bone meal

Meat-and-bone meal containing any sheep or goat protein, or any feedstuffs containing that type of *meat-and-bone meal*, which originate from countries not considered free of scrapie should not be traded between countries for ruminant feeding.

<center>Article 14.9.11.</center>

Recommendations for importation from countries or zones not considered free from scrapie

for skulls including brains, ganglia and eyes, vertebral column including ganglia and spinal cord, tonsils, thymus, spleen, intestine, adrenal gland, pancreas, or liver, and protein products derived there from, from sheep and goats

Veterinary Authorities should require the presentation of an *international veterinary certificate* attesting that:

1.   in the country or *zone*:

    a)   the *disease* is compulsorily notifiable;

    b)   an awareness, *surveillance* and monitoring system as referred to in Article 14.9.2. is in place;

    c)   affected sheep and goats are slaughtered and completely destroyed;

2.   the materials come from sheep and goats that showed no clinical sign of scrapie on the day of *slaughter*.

Recommendations for the importation of ovine and caprine materials destined for the preparation of biologicals

Veterinary Authorities of *importing countries* should require the presentation of an *international veterinary certificate* attesting that the products originate from sheep and goats born and raised in a scrapie free country, *zone* or *establishment*.

Article 14.9.13.

Principles for declaring a country or zone historically free from scrapie

Articles 14.9.13. and 14.9.14. outline principles for declaring a country or *zone* free from scrapie.

An essential prerequisite to provide the guarantees required for the recognition of freedom from *disease/infection* is that the *Veterinary Services* of the Member comply with the provisions of Chapter 3.1. on evaluation of *Veterinary Services*, and, if relevant, with the provisions of Chapter 4.3. on zoning and compartmentalisation.

The provisions of the above-mentioned articles are based on the principles developed in Chapter 1.4. and the following premises:

1. the sheep population of the country or *zone* includes a range of genotypes known to be susceptible to scrapie;

2. the *Veterinary Services* have the competence, capacity and mandate to investigate, diagnose and report scrapie, if present;

3. the absence of scrapie over a long period of time can be substantiated by effective *disease* investigation and reporting by the *Veterinary Services* of an OIE Member.

Article 14.9.14.

Requirements to declare a country or zone historically free from scrapie

A country or *zone* may be recognised free from scrapie without having applied the requirements of Article 14.9.3. when:

a) scrapie has been notifiable for at least 25 years; and

b) a formal programme of targeted *surveillance* and monitoring can be documented as having been in place for at least 10 years; and

c) the presence of a range of scrapie susceptible genotypes in this sheep population can be documented; and

d) appropriate measures to prevent scrapie introduction can be documented as having been in place for at least 25 years; and

    i)   either scrapie has never been reported; or

    ii) no *case* of scrapie has been reported for at least 25 years.

APPENDIX 4

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