Attachment A

AQIS
Protecting our way of life!

IMPORT RISK ANALYSIS REPORT ON THE REVISION OF IMPORT POLICY RELATED TO SCRAPIE

FINAL REPORT

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EXECUTIVE SUMMARY

The Australian Quarantine and Inspection Service (AQIS) has assessed the probability of introducing scrapie via the importation of live sheep and goats from Canada, the United States of America and Member States of the European Union as high. However the probability of introducing scrapie via ovine and caprine embryos and semen has been assessed as low.

The risks associated with importing scrapie via sheep and goats were considered to be unmanageable and AQIS does not propose to establish conditions for importation at this time. Risk management options for the importation of ovine and caprine genetic material are assessed and measures recommended. The risk management measures recommended for ovine embryos and semen include:

- donors must be 5 years of age and older before export of genetic material;
- donors must be genetically susceptible to scrapie;
- donors must be from breeds in which PrP genotypes and their susceptibility to scrapie have been studied sufficiently to permit the accurate selection of scrapie susceptible donors;
- country of export restrictions;
- flock of origin restrictions;
- autopsy of semen donors and female embryo donors and negative immunohistochemical testing for scrapie before export of genetic material;
- embryos collected, stored and handled in accordance with OIE and IETS recommendations;
- semen collected, stored and handled in accordance with OIE recommendations for small ruminants.

The risk management measures recommended for caprine semen and embryos are similar but do not include restrictions based on PrP genotype or breed.

Import requirements relating to scrapie are included in the attached conditions for ovine embryos and semen and caprine embryos and semen from Canada, the United States of America and Member States of the European Union.

ABBREVIATIONS AND ACRONYMS

APHIS	Animal and Plant Health Inspection Service
AQIS	Australian Quarantine and Inspection Service
AQPM	Animal Quarantine Policy Memorandum
BSE	bovine spongiform encephalopathy
CFIA	Canadian Food Inspection Agency
CMP	Complete Monitored Program
CNS	central nervous system
Code	OIE International Animal Health Code
DNA	deoxyribonucleic acid
EU	Member States of the European Union
nvCJD	new variant Creutzfeldt-Jacob disease
NZ	New Zealand
OIE	Office International des Epizooties
PCR	polymerase chain reaction
PrP	prion protein
PrPsc	infective prion protein
RFLP	restriction fragment length polymorphism
RSA	Republic of South Africa
SIA	scrapie infective agent
SFAP	Scrapie Freedom Assurance Program
TSE	transmissible spongiform encephalopathy
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture

1 INTRODUCTION

Scrapie is a progressive and invariably fatal transmissible spongiform encephalopathy (TSE) affecting sheep and goats. Scrapie has been known to occur in the United Kingdom (UK) and Germany since the eighteenth century and is now present in many of the sheep raising countries of the world. Scrapie has been diagnosed in Australia on one occasion - on a single property in Victoria in 1952. Four of a group of 10 Suffolk sheep imported from the UK were affected and the disease was eradicated by slaughter and quarantine.

Since 1952, the importation of sheep and goats into Australia has been prohibited from all countries except New Zealand (NZ). Ovine and caprine genetic material has been imported from NZ and ovine and caprine embryos from the Republic of South Africa (RSA). Both NZ and RSA are considered by Australian Quarantine and Inspection Service (AQIS) to be free from scrapie. There have been three importations of washed ovine and caprine embryos from countries where scrapie is endemic.

The imported embryos were subjected to a Scrapie Freedom Assurance Program (SFAP). SFAPs were comprehensive post-arrival quarantine programs which took at least three and a half years to complete and were designed to ensure detection of the scrapie agent if present in the imported embryos. The animals produced from imported embryos and sentinel animals were held on quarantine premises that met strict design, security and management requirements, and were subject to AQIS supervision. The quarantine program involved control over the movement of personnel, equipment, animal products, live animals, embryos and semen. Surveillance for scrapie included the collection and inoculation of mesenteric lymph node material into sentinel sheep or goats and systematic performance of autopsies on all sentinels and sick or dead animals on the quarantine station. The animals produced from the imported embryos, sentinels and embryo recipients were slaughtered, autopsied and tested for scrapie before the second, and subsequent, generations were released from quarantine.

AQIS required health certification stating that scrapie was notifiable in the country of export and the flock of origin had remained free from scrapie during the 10 year period immediately prior to export of the embryos.

There was one importation of ovine and caprine embryos from the United States of America [USA] (in this instance live animals were imported to the Cocos Island Animal Quarantine Station and their progeny subjected to a SFAP on the mainland), one importation of goat embryos from Zimbabwe and one importation of ovine embryos from Cyprus. Each of these importations were subjected to a SFAP carried out in animal quarantine stations at Torrens Island, Kirra and Terraweena in South Australia; Kununurra and Wongan Hills in Western Australia; and Glendook in Victoria.

There is a growing demand for the importation of ovine and caprine genetic material from North America and the European Union (EU). Importers are hesitant about becoming involved in expensive SFAPs and have hoped that recent developments in the study of prion protein (PrP) genotypes, and the diagnosis of scrapie, would lead to the adoption of simpler import requirements.

AQIS proposed that the quarantine risk associated with sheep and goats in respect of scrapie be the subject of a routine risk analysis in Animal Quarantine Policy Memorandum (AQPM) 1997/67, circulated 17 September 1997. AQIS received comments from 10 respondents; all supported the proposal. One of the respondents stressed that careful consideration should be given to policy relating to biologicals, particularly those intended for *in vivo* use. Two other respondents questioned the use of

a routine risk analysis process stating that significant uncertainties still existed about the quarantine risks associated with scrapie.

Current quarantine policy and practice

Scrapie is listed in Schedule 3 *Diseases affecting animals* of Quarantine Proclamation 1998 and as such is a quarantinable disease. Animal semen, embryos or ova are listed in Table 11 of Part 4, Section 27 as *prohibited biological materials*. Section 28 *Importation of biological materials* states that importation is not prohibited if the Director of Quarantine has granted a permit. Division 2 of Part 6 of the Proclamation deals with the importation of animals, animal parts and animal products into Australia. Section 37 of Division 2 addresses the importation of live animals which are prohibited unless a permit to import has been issued. Section 38 (4) prohibits the importation of animal parts (including animal reproductive material) except by permit.

Importation of sheep and goats is currently prohibited from all countries except NZ. The importation of ovine and caprine semen is permitted only from NZ and ovine and caprine embryos only from NZ and the RSA.

2 HAZARD IDENTIFICATION AND EXPOSURE PATHWAYS

2.1 HAZARD IDENTIFICATION

Scrapie is an Office International des Epizooties (OIE) list B disease that is not present in Australia.

Australia is free from scrapie and the introduction of scrapie infective agent (SIA) is considered to be a hazard in the importation of sheep, goats and ovine and caprine genetic material from countries affected by scrapie.

2.2 EXPOSURE PATHWAYS

SIA could be introduced to Australia in live sheep or goats imported from scrapie endemic countries. The 1952 outbreak of scrapie occurred in sheep imported from the UK.

Despite the efforts of research groups in the USA and Scotland it is still unclear whether scrapie can be transmitted via washed ovine embryos. The most recent publications present conflicting conclusions. Until the situation is elucidated by further research it must be presumed that there is a risk that scrapie can be transmitted by ovine embryo transfer.

The limited amount of published work available on the transmission of scrapie via caprine embryos suggests that transmission does not occur.

Attempts to isolate SIA from ovine semen, seminal vesicles and testes have not been successful and limited research and observations indicate that scrapie is not transmitted in semen. However further research is required to confirm this.

AQIS is not aware of any published information on the transmission of scrapie in caprine semen.

3 RISK ASSESSMENT

Scrapie is a progressive and invariably fatal degenerative disease of the central nervous system of sheep and goats.

The precise molecular nature of the SIA has not been determined. Like a virus it can be transmitted from one host to another but biochemically it does not resemble a virus as it is extremely resistant to heat, irradiation and most disinfectants known to inactivate viruses. When SIA reaches the central nervous system it produces a change of the natural, soluble cell membrane protein PrP into an abnormal, insoluble and protease resistant form - PrPsc . It is the accumulation of PrPsc in the neurones that leads to brain degeneration with the characteristic spongiform changes which are seen histologically in all TSEs (Wrathall 1997).

3.1 PROBABILITY OF SIA BEING PRESENT IN SHEEP AND GOATS

The distribution of SIA within infected sheep has been the subject of much research the results of which are summarised by Wrathall (1997). In an infected, clinically normal 25 month old sheep SIA was found in lymphatic tissue, spleen, ileum and proximal colon and parts of the brain. In naturally infected adult sheep showing clinical signs of scrapie, SIA was found at high levels in the brain and spinal cord; at moderate levels in the spleen, tonsil, ileum, proximal colon and lymph nodes; and at low levels in the cerebrospinal fluid, pituitary gland, distal colon, adrenal cortex and sciatic nerve and occasionally traces were also detected in the nasal mucosa, pancreas, liver, bone marrow and thymus.

The probability of transmission of SIA via placentae is unclear, as there is conflicting evidence regarding the infectivity of placentae from infected ewes (Race *et al* 1998). SIA has not been detected in testes or semen.

Scrapie is endemic and compulsorily notifiable in Canada (from 1945) and the USA (from 1952). The prevalence of the disease is very low in both North American countries with 74 cases reported in Canada and 63 cases in the USA during 1998 (OIE). However, the health status of a flock or herd with respect to scrapie can be difficult to determine. Within flock/herd prevalence varies and is difficult to assess as few tests currently available detect subclinical cases of infection.

There are more than 13 000 flocks in Canada and 90 000 flocks in the USA. On 7 December 1998 there were 63 known infected flocks in the USA, 40 of which were Suffolk sheep (USDA). Scrapie has not been diagnosed in goats in Canada since 1973. At 20 May 1997 only 5 natural cases of scrapie had been diagnosed in goats in the USA (USDA).

Scrapie has been compulsorily notifiable in Member States of the EU since 1993 (Council Directive 91/68/EEC of 28/1/91). Scrapie has been reported in sheep and goats in the UK (465 cases in 1998) and Italy (9 outbreaks in 1998); and sheep only in France (52 outbreaks in 1997), Greece (6 outbreaks in 1998), Germany (2 outbreaks in 1998), Ireland (9 outbreaks in 1998), the Netherlands (16 outbreaks in 1998), Norway (3 outbreaks in 1998), Switzerland (no outbreaks since 1995), Sweden (no outbreaks reported since 1986) and Austria (1 outbreak April 2000).. Scrapie has not been reported from Denmark, Finland, Luxembourg, Portugal or Spain.

There is no test for scrapie that will detect early infection. The incubation period varies from 6 months to 8 years in sheep and 8 months to 2 years in goats. In endemic countries the prevalence in goats is far less than in sheep and scrapie has been reported in goats only in the UK, Italy, Canada (not since 1973) and the USA. There is no evidence that maternal transmission occurs in goats and it appears that goats become infected either by close association with sheep or from eating contaminated meat-and-bone meal.

Recently developed tests will detect scrapie infection at least 12 months before clinical evidence of infection. These tests involve the use of immunohistochemistry to detect PrPsc in lymphatic tissue obtained by biopsy from either the tonsil (Schreuder *et al* 1998) or, the more accessible, nictitating membrane (O'Rourke *et al* 1998).

3.2 PROBABILITY OF SIA BEING IN OVINE AND CAPRINE EMBRYOS

The interpretation of results of research to determine if scrapie can be transmitted via ovine embryo transfer has been complicated by low numbers of susceptible control sheep (Foote *et al* 1993), the use of unwashed embryos and possibly sub-clinically infected recipients (Foster *et al* 1992) and possible exposure of trial sheep to SIA in the environment (Foster *et al* 1996). Despite the considerable amount of research in both Scotland and the USA the probability that scrapie is transmitted by ovine embryo transfer is unknown.

Published information on scrapie transmission by embryo transfer in goats is limited but indicates that scrapie is probably not transmitted via caprine embryos (Wrathall 1997). Foster *et al* 1999 showed that experimentally induced BSE did not transmit via caprine embryos or by maternal transmission to offspring born to infected donors.

3.3 PROBABILITY THAT SIA IS PRESENT IN OVINE AND CAPRINE SEMEN

The limited observations on the transmission of scrapie in ovine semen indicate that scrapie is probably not transmitted in ovine semen (Wrathall 1997). There is no evidence that scrapie is transmitted in caprine semen. There is strong evidence that Bovine Spongiform Encephalopathy (BSE) is not transmitted by bovine semen and this is reflected in the OIE *Code* Chapter 3.2.13. on BSE. Foster *et al* 1999 found no indication that experimental BSE in goats could be spread by venereal infection to males mated with infected does.

3.4 CONSEQUENCES OF SIA INTRODUCTION

Australia's strategy for control and eradication of scrapie, an assessment of social and economic effects, funding and compensation and strategy if the disease becomes established are detailed in AUSTVETPLAN 2nd Edition 1996.

Biological consequences

If infected sheep or goats were introduced it could be several years before the disease became evident. The length of this period would depend on the age and the genotypes of the imported infected animals. If import conditions restricted the numbers imported to small consignments of breeding animals eradication would not be difficult as the animals could easily be traced and

imported animals, offspring and in-contact animals destroyed. However if large numbers of animals of various breeds were imported eradication may be difficult.

The consequences of the introduction into Australia of SIA in genetic material would depend on several variables:

- The number of recipients
- The genotype of the recipients
- The management of the recipients

If ewes could be infected by insemination the worst case scenario could be the importation of infective semen from one or more donors. This semen could be used to impregnate and infect females in many flocks in many parts of the continent. The introduction may not become apparent for many years, depending on the PrP genotype of the infected sheep and it would be unlikely that clinical disease would be seen before 2 or 3 years after importation. If the animals produced were keenly sought by studs and breeders as superior genetic stock, infected offspring could be numerous and widespread by the time scrapie was diagnosed. However even in this worst case scenario the eradication of the disease would be simplified by the fact that the breeds of sheep affected would be known and the tracing of the imported infected semen and the offspring not unduly difficult.

Environmental consequences

Australia has no native sheep or goats and the environmental impact of the introduction of scrapie would be negligible. Large populations of feral goats do occur in some areas of Australia. However it would be most unlikely that scrapie would be maintained in these populations.

Economic consequences

If scrapie was to become established in commercial flocks, it is unlikely that the disease would have a significant economic impact on Australia's livestock industries. Scrapie is endemic in the flocks of our main trading partners and only scrapie free countries such as New Zealand, the RSA and South American countries – which are all exporters, not importers, of sheep and ovine products – would be likely to apply any trade restrictions. The most significant effect of the establishment of scrapie would be the implications for Australia's TSE-free status, and possible effects on exports of some ruminant products, such as ovine pharmaceutical products for *in vivo* use. Exports of such products could be greatly reduced at least until the extent of the outbreak was determined and regionalisation applied, if possible.

The establishment of scrapie would be unlikely to have significant impact on production, as even in countries where the prevalence of scrapie is high, production is not greatly affected. If the disease were to become established, transmission would probably be strictly limited due to factors associated with Australia's pastoral management system. The prevalence of infection would be expected to remain extremely low and infection could well be confined to certain geographic areas.

The most significant economic effect of the establishment of scrapie would probably be the cost of eradication (see AUSTVETPLAN 2nd Edition 1996).

3.5 CONCLUSIONS

Scientific evidence indicates that the probability of importing scrapie via live sheep and goats is far greater than that associated with the importation of embryos or semen. Factors contributing to the higher probability associated with live animals include the lack of a test to detect animals incubating the disease until 12 – 18 months prior to the development of clinical signs, and the difficulty of accurately ascertaining flock or herd status for this pathogen. Until these problems are solved, it is not possible to develop conditions that would achieve Australia's appropriate level of protection for the importation of live sheep and goats from countries/zones affected by scrapie.

There is conflicting published data on the transmission of scrapie by ovine embryo transfer however those involved in research in this field agree that appropriate embryo collection, handling and transfer techniques reduce the risk significantly. The risks associated with *in vivo* –- derived frozen embryos would appear to be less than those associated with *in vitro* – derived embryos. The risks associated with the importation of embryos from scrapie affected countries can be managed by the application of appropriate measures for the collection, handling and storage of embryos from selected donors.

There is some evidence that the probability of importing scrapie in ovine and caprine semen is less than that associated with embryo transfer and this is supported by results obtained in practice. However this evidence is not sufficient to warrant the adoption of less restrictive conditions in relation to flock/herd health certification, or the selection of semen donors, than that applied to donors of embryos.

The risk of importing scrapie in ovine embryos and semen is probably far greater than the risk of importing scrapie in caprine embryos or semen.

In the worst case scenario, the establishment of SIA may not become evident for 2-3 years, by which time infected sheep could have been moved far and wide. However, these sheep would be of known breed and could fairly readily be traced to the studs and major producers that had purchased them. It is considered that the economic impact of the establishment of scrapie would not be great, but the implications for Australia's TSE-free status would be of concern and exports of ovine products for pharmaceutical uses could be reduced on a temporary or longer term basis.

4 RISK MANAGEMENT - OVINE SEMEN AND EMBRYOS

Since the early 1980s, the importation of sheep and goat embryos from scrapie-affected countries has been permitted if the embryos were subjected to a SFAP. SFAPs were expensive and time consuming, and involved the maintenance of a large number of animals in a quarantine station for a minimum period of 3.5 years. In light of the findings of this import risk analysis, AQIS has concluded that ovine semen and embryos could be safely imported using less trade restrictive policies. Appropriate risk management measures are described in this Section.

Risk management measures considered:

- selection of donors based on:
  - history of flock of origin;
  - susceptible genotype for each breed, and
  - age - animals should be beyond the age when most animals of the genotype show clinical evidence of infection.
- testing of donors (in order of descending sensitivity) using:
  - autopsy of donors, following collection, and testing of central nervous system, lymphatic and other tissues, or
  - testing of mesenteric lymph nodes of female donors, or
  - tonsil biopsy test, or
  - nictitating membrane test.
- handling, processing and storage of semen and embryos in accordance with the relevant OIE Animal Health *Code* requirements.

Selection of embryo and semen donors based on the history of the flock of origin, susceptible genotype for each breed and age of the donor reduces the probability of using a scrapie infected donor. For the reasons given in this section, these measures, even when combined with handling, processing and storage of semen and embryos in accordance with relevant OIE requirements do not reduce the risk to a level which can achieve Australia's appropriate level of protection (ALOP). Testing of donors using the most sensitive techniques (autopsy of donors after collection and testing of central nervous system, lymphatic and other tissues), alone or in combination with *Code* recommended standards for semen and embryos, also considerably reduces the probability of using a scrapie infected donor, but not to the required level. As explained below, testing of specific tissues can detect infected animals at certain (relatively advanced) stages of infection. However, the timeframe/course of tissue distribution of SIA is not sufficiently well understood and testing cannot detect animals in the early stage of infection.

Each of the measures described above will reduce the probability of collecting infected embryos or semen but no single measure or subgroup of measures would achieve Australia's ALOP. However, on the basis that the new measures, in combination, meet the ALOP and are significantly less trade restrictive than the current conditions (based on SFAP) AQIS proposes to apply them to the importation of sheep embryos from Canada, the USA and Member States of the EU.

4.1 HISTORY OF SCRAPIE IN THE FLOCK OF ORIGIN

To ascertain the probability of an animal being infected with scrapie a reliable history of the health of the flock of origin is essential. The OIE *Code* (draft Chapter 3.3.8. January 1999) on scrapie states that a flock may be considered to be *scrapie free* if:

1 in the country the following conditions are fulfilled:

- *the disease is notifiable and an effective and continuous national surveillance system as referred to in Article 3.3.8.1. is practised;*
- *affected sheep and goats as well as their progeny are slaughtered and completely destroyed;*
- *the feeding to sheep and goats of meat-and-bone meal derived from ruminants originating from countries not free from animal TSE has been banned and effectively enforced for at least 5 years;*
- *an official accreditation scheme is in operation under the supervision of the Veterinary Administration;*

2 in the flock the following conditions are fulfilled:

- *animals should be permanently identified, to enable trace back to their dam and flock/herd of origin;*
- *records of parentage and movements in animals in and out of the flock are established and maintained;*
- *introductions of animals are allowed only from flocks of the same status;*
- *an official veterinarian inspects the flock at least once a year;*
- *no case of scrapie has been confirmed for at least 6 years;*
- *no co-mingling with flocks of a lower status is permitted;*
- *all culled animals over 18 months of age are inspected, and a proportion of them representing at least 1% of the number of the breeding animals present in the flock/herd are tested in the laboratory for scrapie annually.*

In response to concerns about the possible links between scrapie and BSE, many countries have initiated programs to confirm the freedom, or accredited freedom, of sheep and goat flocks or herds. These are voluntary programs and have been operating for a few years only.

USA

The voluntary scrapie certification program in the USA operates under the auspices of the USDA and commenced in October 1992. Revised standards were adopted 1 July 1999 as Voluntary Scrapie Flock Certification Standards. There are two program categories:

1. Complete Monitored Category, and
2. Selective Monitored Category – this category is open to any flock but is mainly intended for slaughter-lamb producers wishing to have an additional method of scrapie surveillance in large production flocks.

An *Enrolled Flock* is one which has been approved to participate in the program. A *Certified Flock* is an *Enrolled Flock* which has participated in the Program for more than 5 years and has met the necessary requirements to progress beyond Enrolled status. Under Program Requirements:

The flock owner or manager who participates in the Complete Monitored category will agree to:

1. *Immediately report scrapie-suspect animals and animals suspected of other neurologic and chronic debilitating (prolonged wasting) illnesses to a State or Federal animal health official or an accredited veterinarian.*
2. *Ensure that proper tissue samples are collected and submitted for diagnostic purposes. Such animals shall not be used for breeding or be disposed of without the prior approval of a State or APHIS representative.*
3. *Officially identify all animals 1 year of age or older within a flock. Officially identify all acquired animals prior to commingling with the flock. All animals less than 1 year of age will be officially identified when a change of ownership occurs, with the exception of those moving within slaughter channels. Official identification for the specified animals will be*
   - *Permanent,*
   - *Secure,*
   - *Unique (assigned from a central repository), and*
   - *Traceable.*

   The following are types of Program-approved identification:
   - *Tamper-resistant ear tag,*
   - *Flank or ear tattoo, and*
   - *Electronic identification.*

   In the case of goats registered with the American Dairy Goat Association, the tattoo may be applied at the tail web.

   A secondary form of identification may be maintained at the owner's discretion.

4. *Maintain records in accordance with the following:*

   Records must be kept for a minimum of 5 years after an animal dies or has otherwise been removed from the flock.

   The following records must be kept on animals present in the flock at the time of initial participation:
   - *Official and any secondary identification number*
   - *Sex*
   - *Breed*
   - *Disposition – date and cause of death, if known, or movement date and to whom;*
   - *Progeny's official and any secondary identification numbers and sex, and*
   - *If available, date of birth or date of acquisition, flock of origin and date of entry and sire and dam's official and any secondary identification.*

   The following records are to be kept on acquired or natural additions to the flock subsequent to enrolment:
   - *Official and any secondary identification number*
   - *Sex*
   - *Breed*
   - *Date of birth or date of acquisition*
   - *The flock of origin and date of entry*
   - *Disposition – date and cause of death, if known, or movement date and to whom*
   - *Sire and dam's official and any secondary identification numbers, and*
   - *Progeny's official and any secondary identification numbers and sex.*

5. *Allow breed associations and registries, livestock markets and packers to disclose records to APHIS representatives or State animal health officials. These records will be used to trace a source of exposure and other exposed animals.*

6. *Notify the State Scrapie Certification Board about acquisitions that would lower the status, status date, or both, of a flock as per part III, section A5c, within 30 days after the animal enters the flock.*

7. *Make animals and records available for inspection by APHIS representatives, State animal health officials and State Scrapie Certification Board representatives, given reasonable prior notice. The owner shall agree to have the necessary facilities and personnel available to assist in inspecting the identification of each animal and the records.*

8. *Ensure that tissues from scrapie-suspect animals, and animals suspected of other neurologic and chronic debilitating (prolonged wasting) illnesses will be submitted to an official laboratory in accordance with part IV and appendix 1. Other tissues will be submitted at the request of the State Scrapie Certification boards or State or Federal animal health official.*

There are also requirements for annual inspections and procedures following the discovery of scrapie in an enrolled flock. Program requirements also include detailed procedures covering the acquisition of animals, commingling, the use of semen and embryos and imported animals from foreign countries.

Canada
The Canadian Food Inspection Agency (CFIA) is in the process of designing a national voluntary scrapie flock certification program (Ron Rogers *pers comm*).

European Union (EU)
There are no EU requirements for scrapie flock accreditation programs, however, Council Directive 91/68/EEC lays down conditions for the importation of breeding sheep into the EU. Under Article 6 (b) with regard to scrapie, they must:

1 *come from a holding satisfying the following requirements:*
   - *the holding is subject to official checks in accordance with Article 3 (1) (b) of Directive 90/425/EEC,*
   - *the animals must be marked,* (= identified)
   - *no case of scrapie has been confirmed for at least two years,*
   - *checking by sampling must be carried out at slaughter on old ewes, intended for culling coming from that holding, except where that holding is situated in a region or a Member State benefiting from conditions to be adopted in accordance with Article 8,*
   - *female animals may only be introduced into that holding if they come from a holding which complies with the same requirements;*

2 *have been continuously kept on a holding or holdings complying with the requirements laid down in 1 since birth or for the last two years;*

The current scrapie monitoring arrangements in the UK do not form the basis of a control or eradication scheme but are in place to support export certification of breeding sheep and goats to

other Member States and accord with Council Directive 91/68/EEC. The Ministry of Agriculture, Fisheries and Food (MAFF) operates a voluntary certification scheme whereby producers can demonstrate, on an annual basis, compliance with the monitoring requirements of Council Directive 91/68/EEC (JM Scudamore *pers comm*). Many flocks/herds which have been in such a voluntary certification scheme for a number of years would probably meet the requirements of the draft OIE Chapter for a *scrapie free* flock.

AQIS considers that the USDA Voluntary Scrapie Flock Certification Program Standards, July 1999 provides equivalent biosecurity to that provided by the requirements in the draft *Code* Chapter. However Council Directive 91/68 provides less biosecurity as it requires that there has been no confirmation of disease for 2 years, compared with 6 years in draft Code Chapter and 5 years in the USDA voluntary program.

The weakness in using the free flock requirement in import conditions is that a free flock may be only a fence line away from an infected flock. The *"no co-mingling"* requirement in the *Code* definition is difficult to certify as it would be unlikely that a stock owner, into say, his third year of a free flock program, would declare that commingling had occurred after local floods had washed away fences, or that one or two neighbour's sheep had been found boxed up with his flock at shearing.

Conclusions

Requiring that the donors come from flocks or herds certified under the USDA Voluntary Scrapie Flock Certification Program or that meet the draft *Code* requirements for freedom from scrapie or equivalent flock status, reduces, but does not eliminate, the risk of using an infected donor.

4.2 SUSCEPTIBILITY OF GENOTYPE

The genotype of the host influences susceptibility to scrapie. It has been shown that the incubation period and other characteristics of scrapie in sheep are controlled by at least two or three polymorphisms located on separate codons within the PrP gene. This gene is present in all mammals and codes for the production of PrP. The gene can be extracted from blood and other tissues and amplified by using polymerase chain reaction (PCR) in combination with restriction fragment length polymorphism (RFLP) to read the deoxyribonucleic acid (DNA) coding sequence. The sheep PrP gene produces a protein of 256 amino acids, each of which is encoded by three DNA bases (ie one codon) in the gene. With respect to risk of expressing the signs of scrapie, at least five variant alleles have been identified as specified by variation (polymorphism) in the amino acids encoded at codons 136, 154 and 171. These five alleles are: $A_{136}R_{154}Q_{171}$ (ARQ); $A_{136}R_{154}R_{171}$ (ARR); $V_{136}R_{154}Q_{171}$ (VRQ); $A_{136}H_{154}Q_{171}$ (AHQ) and $A_{136}R_{154}H_{171}$ (ARH) where A = alanine, H = histidine, Q = glutamine, R = arginine and V = valine, respectively (Dawson *et al* 1998).

Genotypes are often represented as three pairs of letters indicating the amino acids encoded on both alleles at each of the three positions. However it is simpler to list the pair of alleles which constitute the genotype. Thus the genotype resulting from the pairing of ARQ and VRQ alleles is given as ARQ/VRQ instead of AV/RR/QQ. The following information and tables are taken from Dawson *et al* 1998:

TABLE 1: Distribution of PrP alleles in different sheep breeds - showing the predominant allele followed by the breeds:

ARQ, ARR
Cotswold, Hampshire, Soay, Suffolk*, Vendeen

ARQ, ARR, VRQ
Bleu du Maine, Border Leicester, Charollais *, Poll Dorset, Wensleydale

ARQ, ARR, AHQ
Bluefaced Leicester

ARQ, ARR, AHQ, VRQ
Cheviot, Dalesbred, Herdwick, Scottish Blackface, Shetland, Swaledale, Welsh Mountain

ARQ, ARR, AHQ, VRQ, ARH
Texel, Lleyn

NB In this table alleles have been listed for the breeds for which there is substantial data , but this information should not be regarded as definitive for the breeds given. Future modification may be required.
* ARH has been found in a small proportion of sheep of these breeds.

Studies of genotypes linked to scrapie in various breeds has shown that scrapie occurs most often in certain genotypes and less frequently, or rarely, in others. The specific mechanism which causes these variations is not known but it has been shown that the major influence is on the length of incubation period. Susceptible genotypes have a short incubation period but the incubation period in other genotypes may be so long that the disease is rarely, if ever, seen (Bossers *et al* 1996). The variation between breeds may be linked to the varying prevalence of different strains of scrapie in different breeds. Studies in both sheep and mice have shown that, following infection, SIA replicates and persists in peripheral (extraneural) tissues but clinical signs only occur when the agent spreads to the central nervous system (CNS). There is some evidence that certain strains of SIA may not spread to the CNS of sheep with a certain PrP genotype. However these animals may retain SIA in peripheral tissues and remain infective and could be a source of environmental and/or transplacental contamination (Wrathall 1997).

Dawson *et al* (1998) reported on details of the genotypes recorded for those breeds for which there is substantial data, grouping them according to the anticipated level of risk of disease in individual sheep and their progeny (Tables 2 to 6). The authors state - “It should be emphasised

that the interpretations of the gene classes are based on probability and not certainty". In the tables the gene classes have been based on an assessment of clinical scrapie risk (R) and scored R1-R5 with the following descriptions:

R1 very low risk.
R2 low risk in individuals and first generation progeny.
R3 low risk in individuals but some progeny may be at risk depending on genotype of other parent.
R4 scrapie occasionally recorded.
R5 sheep at greatest risk from scrapie.

TABLE 2 for breeds with the ARQ and ARR alleles (and occasionally ARH), for example the Suffolk, Hampshire Down, Soay and Cotswold * (scrapie is rarely, if ever, seen in Cotswold)

R1	RR
R3	RQ, RH, HH
R5	QQ, QH

*With this group the only variation is at Codon 171 and it is accepted practice that the genotype be represented at that position only.

TABLE 3 for breeds with ARQ, ARR and VRQ alleles, for example the Charollais, Border Leicester, Poll Dorset, Wensleydale and the Bleu du Maine.

R1	ARR/ARR
R3	ARR/ARQ
R4	ARQ/ARQ, ARR/VRQ
R5	ARQ/VRQ, VRQ/VRQ

TABLE 4 for the Bluefaced Leicester (ARQ, AHQ and ARR alleles)*

R1	ARR/ARR
R2	ARR/AHQ, AHQ/AHQ
R3	ARR/ARQ, ARQ/AHQ
R4	ARQ/ARQ

* This assessment of susceptibility is theoretical as there is little, if any, evidence of scrapie in the purebred.

TABLE 5 for breeds with the ARQ, ARR, VRQ and AHQ alleles, for example the Blackface, Cheviot, Dalesbred, Herdwick, Shetland, Swaledale and Welsh Mountain.

R1	ARR/ARR
R2	ARR/AHQ, AHQ/AHQ
R3	ARR/ARQ, ARQ/AHQ
R4	ARR/VRQ, AHQ/VRQ, ARQ/ARQ
R5	ARQ/VRQ, VRQ/VRQ

TABLE 6 for breeds with ARQ, ARR, VRQ, AHQ and ARH, eg Texel, Lleyn*

R1	ARR/ARR
R2	ARR/AHQ, AHQ/AHQ
R3	ARR/ARQ, ARR/ARH, ARQ/AHQ, AHQ/ARH
R4	ARH/ARH, ARQ/ARH, ARQ/ARQ, ARR/VRQ, AHQ/VRQ
R5	ARQ/VRQ, ARH/VRQ, VRQ/VRQ

* as the influence of the ARH allele is not properly understood, this information must be interpreted with care.

Several other authors have published data on breed genotypes which were not included in Dawson *et al* (1998):

- Bossers *et al* (1996) determined the PrP genotype and lifespans of over 50 Flemish and Swifter sheep and reported that 83 per cent of sheep homozygous to VQ allele at codons 136 and 171 died at a mean age of 25 months. The development of scrapie was delayed or did not develop in sheep heterozygous for VQ and sheep with at least one AR allele including VQ/AR sheep did not develop scrapie.
- Hunter and Cairns (1998) reported that PrP genotypes susceptible to scrapie were found in merinos from UK and Australia. The highly susceptible genotype VRQ/ARQ was found in 1 of 39 Australian merinos and the susceptible genotype ARQ/ARQ was found in 49 percent of UK merinos and 46 percent of Australian merinos.

There is an obvious dilemma when considering the use of the PrP genotype as a tool to reduce the risk of introducing SIA in ovine genetic material. The authorities of countries in which scrapie is endemic can see the advantage of selection for genotypes which are not susceptible to scrapie. Clinical scrapie would be rarely seen and might disappear if the vast majority of sheep were of non-susceptible genotypes. However, as non-susceptible sheep may be capable of carrying, and transmitting SIA to susceptible sheep, it would be unwise for a scrapie free country, such as Australia, to base its risk management regime solely on the importation of genetic material from non-susceptible genotype donor animals.

Conclusions

The probability of collecting embryos or semen donors from a scrapie infected donor would be considerably reduced if donors are of a susceptible genotype and

older than the age at which animals of this particular genotype would normally show clinical evidence of scrapie.

4.3 THE INTERACTION BETWEEN SCRAPIE STRAIN, PRP GENOTYPE AND SCRAPIE INCUBATION PERIOD

The study of scrapie has been hampered by its long but variable incubation period complicated by differing genetic susceptibility within and between breeds of sheep. Detailed research into the nature of scrapie was facilitated by infecting mice with scrapie infective agent isolated from sheep or goats. It was found that some of these isolates varied in that they produced incubation periods of different lengths and different brain pathology to other isolates. These varying isolates were called "strains" of scrapie infective agent. To counter between-species barriers, and contamination, many of these "strains" were passaged through mice, hamsters or sheep by intra-cranial injection.

From Prusiner (1996) – "…studies in mice demonstrated the existence of many scrapie "strains", where extracts prepared from the brains of mice inoculated with a particular preparation of prions produced a similar disease in inoculated recipients. While the clinical signs of scrapie for different prion isolates in mice tended to be similar, the isolate could be distinguished by the incubation times, the distribution of CNS vacuolation that they produced, and whether or not amyloid plaques formed…The length of incubation times have been used to distinguish prion strains inoculated into sheep, goats, mice and hamsters."

From Wrathall (1997) – "there are approximately 20 phenotypically distinct strains of scrapie…(which) fall into two groups, A and C, and this classification depends on their interaction with different genotypes of mice and sheep. The so called SSBP-1 ('sheep scrapie brain pool') inoculum, originally used in 1950 and still used in research, including in the sheep embryo transfer experiments …is thought to contain a mixture of at least three A strains. Currently only one scrapie strain, CH1641, is known to behave as a group C strain…"

Hope et al (1999) classified PrP in brain material from natural and experimental cases of scrapie by calculating the percentages of different glycoforms. Three distinct biological groups were identified and classified as the following three strains:

A some natural scrapie and the SSBP/1 isolate
B natural scrapie, and
C CH1641 and BSE.

It is difficult to determine the relevance of strains, or infection with passaged isolates, when assessing risk management measures for natural scrapie. Natural scrapie may involve many strains and produce a quite different pathogenesis to that produced by artificial infection with a passaged isolate in sheep with known PrP genotypes (Hunter *et al* 1996).

CH1641 isolate of scrapie

CH1641 was isolated from a natural case of scrapie in a Cheviot sheep from a flock selected for short incubation periods for scrapie. From Foster and Dickinson (1988) – "The CH1641 isolate has been characterised by serial passage in sheep. It is either a single strain of scrapie or an unresolved mixture of strains. The changes in incubation periods of the CH1641 isolate at second and third passage are different from those of SSBP/1…and those of other strains of scrapie at first

passage in sheep. These incubation properties of the CH1641 isolate have led to its classification as a C group strain…"

Intracerebral injection of CH1641 into sheep from the short incubation period ("positive") line of Cheviots produced the following incubation periods (days):
1st passage 392 2nd passage 856 3rd passage 595
Intracerebral injection into sheep from the long incubation period ("negative") line of Cheviots produced the following incubation periods (days):
1st passage 751 2nd passage 360 3rd passage 360.

The material used in the second and third passages was obtained from a terminally sick sheep from the long incubation period ("negative") line of Cheviots.

These incubation periods indicated that, at least during the second and third passage, CH1641 produced long incubation periods in sheep which would have produced a short incubation period after infection with SSBP/1 and short incubation periods, during the second and third passage, in sheep which would have produced long incubation periods after SSBP/1 infection. These results were difficult to explain at the time of the research when it was believed that the incubation period of scrapie in sheep was controlled by a single gene, *Sip* (scrapie incubation period), with two alleles – sA (short incubation period) and pA (long incubation period).

From Wrathall (1997) – "…it is known that incubation period and other scrapie characteristics in sheep are controlled not in simple Mendelian fashion by a single polymorphism (ie the *Sip* gene), but by at least 2 or 3 distinct polymorphisms located on separate codons within the PrP gene. This gene, which is present in all mammals, codes for the production of PrP protein and there is good evidence that the *Sip* and *Sinc* genes in sheep and mice respectively, are in fact the same as the PrP gene."

Subsequent work with the CH1641 isolate (Goldmann *et al* 1994) went a long way towards explaining the apparent aberrant behaviour of this isolate. It was found that susceptibility to CH1641 was influenced largely by PrP genotypes homozygous for glutamine (QQ) at codon 171 whereas the presence of arginine (RR) at codon 171 prolonged the incubation period. Dimorphism at codon 136 had a minor effect on incubation period in that Valine (V)136 lengthened the incubation period when heterozygous but shortened the incubation period when homozygous indicating that a polymorphism barrier influenced incubation periods. The authors also noted that the effects of PrP genotype on the disease produced were similar following artificial infection with either CH1641 or BSE agent.

In vitro research with sheep PrP (Bossers A *et al* 1997) indicated that scrapie susceptibility is not only determined by the PrP genotype of the acceptor animal but also by the PrP genotype of the animal that produced the infectious PrPsc. The authors postulated that this hypothesis could explain the behaviour of CH1641 which was initially isolated from a short incubation period ("positive") line of Cheviot sheep (VQ). When CH1641 was initially passaged it produced a short incubation period in the "positive" line Cheviots and a long incubation period in the "negative" line Cheviots. However subsequent passages from material isolated from a "negative" line Cheviot produced long incubation periods in "positive" line and short incubation periods in "negative" line Cheviots. It was hypothesised that this effect was probably due to the polymorphism barrier.

Natural scrapie

Experimental scrapie using single isolates has revealed much about the pathogenesis of the disease but import risk management measures must be assessed against natural scrapie. There have been several studies on the influence of PrP genotype in natural scrapie outbreaks. An examination of these studies could indicate if scrapie strains influenced the pathogenesis or epidemiology of the disease.

Hunter N *et al* (1993) reported on a study of natural scrapie in a flock of Swaledale sheep and concluded that scrapie susceptibility and short incubation periods was associated with the presence of V-136, whereas alanine(A)-136 was associated with long incubation periods and absence of scrapie.

Laplanche JL *et al* (1993) also reported that the presence of V-136 was linked with scrapie in Romanov and Ile-de-France sheep. They also reported polymorphisms at codons 112, 154 and 171. The relationship between V-136 and the occurrence of natural scrapie in several breeds of sheep was analysed by Hunter N *et al* (1994). V-136 was found in 91-100% of scrapie sheep of the Shetland, Scottish Halfbreed and Bleu du Maine breeds however V-136 was not associated with the occurrence of scrapie in Poll Dorsets or Suffolks. Data was also presented on the age of scrapie affected sheep and their 136 codon genotypes. Although the short incubation periods were similar for both VV and VA genotypes the longer incubation periods varied markedly with 4 years being the longest recorded for VV but 8 years being the longest for a VA sheep.

Onadera A *et al* (1994) reported that the occurrence of scrapie in mainly Suffolk sheep in Japan was related to PrP genotypes and that the prevalence of these genotypes varied from district to district. Westerway *et al* (1994) reported that the occurrence of scrapie in Suffolk sheep in the USA was closely correlated to the homozygous QQ - 171 of the PrP gene. Clouscard *et al* (1995) reported on an outbreak of scrapie in a flock of Romanov sheep in which 60 sheep (81%) died over a 14 month period, the age at death varied from 687-968 days (median 726). Most scrapie affected animals were QQ at 171 particularly in the Lacaune breed in which codon 136 had limited polymorphism. QH (histidine) 171 sheep were as susceptible as QQ but sheep with R-171 were resistant to infection (RQ, RH and RR).

Belt PBGM *et al* (1995) identified 5 different polymorphisms at codons 136, 154 and 171 in Texel sheep. The allelic variants were designated VRQ, ARR, ARQ, ARH and AHQ. The VRQ variant was associated with scrapie infection and ARR with scrapie freedom. ARQ and ARH were found in both groups in equal frequency. Allelic frequencies were studied in both scrapie affected Suffolk and non-affected sheep in the USA (O'Rourke *et al* 1996). All 30 affected sheep from a group of 575 were QQ 171 but this same genotype was detected in 56 % of clinically normal sheep. None of the scrapie affected sheep had valine at codon 136.

Bossers *et al* (1996) studied an outbreak of scrapie in a mixed flock of Flemish and Swifter sheep. 83% of the scrapie affected sheep were VQ/VQ and all died from scrapie before 2 years old. Sheep heterozygous for VQ showed a longer incubation period or did not develop scrapie. Sheep with at least one AR allele including VQ/AR did not develop scrapie.

Hunter N *et al* (1996) reported on an outbreak of natural scrapie in a Cheviot sheep flock that had been free from the disease. Of the sheep that died from scrapie 77% were VV 136 and 23% VA 136. At the beginning of the outbreak scrapie was seen in some older sheep but once established the age at death of VV 136 sheep ranged from 700-900 days (2-2.5 years) and 1100-1200 days

(3-3.3 years) for VA 136 sheep. From the beginning of the outbreak no VV 136 sheep lived longer than 971 days. Polymorphism at codons 154 and 171 also influenced the susceptibility of VA 136 genotypes. Most VQ/AQ succumbed but VR/AQ were apparently resistant. There was also some survival advantage with HR 154 rather than RR 154 genotype.

Hunter N *et al* (1997a) reported on natural scrapie in a Suffolk flock in Scotland. About 1000 sheep were genotype tested and scrapie was only seen in sheep with QQ and QR alleles at codon 171 but was not seen in sheep with QH, RH or RR alleles at codon 171. No detail was given to relate genotype with age at death.

Hunter N *et al* (1997b) examined the relationships of 11 different genotypes and susceptibility to scrapie in over 400 sheep of the following breeds – Bleu du Maine, Herdwick, Merino x Shetland, Poll Dorset, Scottish Halfbreed, Shetland, Soay, Suffolk and Swaledale in Britain. Scrapie was always associated with QQ 171 and Valine-136 where they occurred. In those breeds where it occurred histidine-154 reduced susceptibility. Sheep with VRQ/VRQ genotype were most susceptible to scrapie in the following breeds – Scottish Halfbreed, Herdwick, Shetland, Bleu du Maine and Swaledale. Sheep with VRQ/ARQ were the most susceptible in the Merino x Shetland and Poll Dorset flocks whilst Suffolk and Soay sheep with ARQ/ARQ were the most susceptible.

Scrapie caused major losses in German flocks during the nineteenth century but as few as eight outbreaks have occurred during the last 50 years (Junghans F 1998). This low incidence is attributed to the strict German policy of completely destocking all infected and in-contact flocks. Of the 8 outbreaks which have occurred since 1973 all but 3 occurred in Suffolks. PrP genotype data was collected from 5 of these outbreaks and all of the diagnosed cases were ARQ/ARQ.

Tranulis MA (1999) reported on the PrP genotypes of scrapie affected and healthy sheep in Norway. More than 90.6% of all scrapie cases were recorded in a Cheviot related types of cross bred sheep called Rygja and Steigar. Like Cheviots a high proportion of these sheep carried Valine-136 and 68.8% (22) of the scrapie affected sheep were homozygous VV136 and 15.6% (5) were heterozygous VA136. There was no significant difference in the mean age of these two groups of scrapie affected sheep (VV136 – 39.4 months and VA136 – 35.2 months) but in the VV136 group the two oldest animals were 6 years old and 3 were 5 years old at the time of death. All natural cases of scrapie were homozygous at codon 154.

None of the authors of the above reports speculated that the strain or strains of scrapie influenced the pathogenesis or epidemiology of the outbreaks.

Scrapie strains and incubation periods

The effects of scrapie strains on the length of incubation periods is complex and is affected by host genetic polymorphisms. Thus the incubation periods with certain rare strains, eg. CH1641 (and with BSE) may be long in some sheep which are of susceptible genotype (ie. those expected to have short incubation periods with other scrapie strains). Nevertheless, short incubation periods are the general rule in homozygous susceptible genotype sheep affected with natural scrapie.

Lateral transmission can also influence the age at which clinical symptoms appear in some sheep. Lateral transmission from infected sheep to susceptible adult sheep may be an infrequent occurrence but does occur. Even infected flocks with a high prevalence frequently include

unaffected susceptible adult sheep (O'Rourke *et al* 1996, Hunter and Cairns 1998) which, presumably, could become infected if appropriately exposed to infective agent. Infection of adult sheep could account for the occasional apparently long incubation period observed in natural scrapie, particularly in recently infected flocks.

Conclusions

- **Most infected sheep with a highly susceptible PrP genotype die of scrapie before 4 years of age**
- **Highly susceptible PrP genotype sheep almost invariably have short incubation periods with natural strains of scrapie**
- **Sheep that are homozygous for susceptible PrP genotypes consistently have short incubation periods following infection with all assessed strains of scrapie and BSE infective agent**
- **Lateral transmission to adult susceptible sheep could account for some apparently long incubation periods.**

4.4 AGE OF DONOR

Maternal transmission plays a major role in many scrapie outbreaks but the exact method of transmission has not been determined. SIA is readily detected in foetal membranes and transplacental transmission is thought to occur. Other evidence indicates that the neonate is infected orally; prolonging the time to weaning in affected flocks increases the incidence of scrapie. Lateral spread is probably most significant when lambing occurs in enclosed spaces and animals are continually exposed to infective material (Kimberlin 1991).

In the vast majority of cases infection occurs at or near birth and the age at death of animals with susceptible genotypes is equivalent to the incubation period of less than 4 years.

Conclusion

If genetic material for export was collected only from susceptible donors over 5 years of age the probability of collecting from a scrapie affected animal would be considerably reduced.

4.5 TESTING OF DONORS

There is evidence that PrPsc is an essential part of SIA and that PrPsc deposition precedes the formation of scrapie lesions in the CNS. Numerous studies have confirmed PrPsc as a sensitive and specific marker for scrapie (Prusiner 1996). PrPsc is detected by immunochemical techniques such as Western blotting or immunohistochemistry. The latter has become an accepted diagnostic tool for diagnosing TSEs and is less cumbersome than Western blotting (Schreuder *et al* 1998). Immunohistochemistry detected PrPsc in the spleens, retropharyngeal lymph nodes, mesenteric lymph nodes and palatine tonsils of all but one of 55 naturally infected sheep (98 percent) confirmed histologically with scrapie at postmortem. PrPsc was detected in the palatine tonsils of 93 percent of sheep with scrapie. The greatest concentration of PrPsc was found in the palatine tonsils of scrapie affected sheep where more than 80 percent of the follicles stained positive PrPsc (Keulen *et al* 1996).

PrPsc has been detected in the lymphoid tissue in naturally infected scrapie sheep as early as 10 to 14 months of age. PrPsc was detected by immunohistochemistry in samples of palatine tonsil obtained by biopsy from naturally infected susceptible homozygous (VRQ/VRQ) sheep at 8 months of age and in heterozygous sheep (VRQ/ARQ) at 14 to 15 months of age (Schreuder *et al* 1998). PrPsc was not detected in the palatine tonsils from sheep with intermediate susceptibility to scrapie (VRQ/ARR) during this period. The authors concluded that, in sheep with susceptible genotypes, PrPsc could be detected in the palatine tonsils at between one-third and one-half of the incubation period, more than eighteen months before clinical signs would be expected to occur in these genotypes.

The authors stressed that a negative PrPsc tonsillar biopsy does not imply that an animal is free from infection as the biopsy could have been taken before SIA reached the palatine tonsils.

Immunohistochemistry was used to detect PrPsc in the readily accessible lymphoid tissue of the nictitating membrane excised from sheep under local anaesthetic (O'Rourke *et al* 1998). The assay was applied to sheep from 11 flocks with a history of scrapie. Staining was seen in the lymphoid tissue from 9 clinically affected animals and a further 16 unaffected sheep, 6 of which subsequently developed clinical signs two to seven months after sampling. Seventeen of the 25 sheep with positive staining were assessed for PrP genotype and were all found to carry the susceptible genotypes AQ/AQ or AQ/VQ. The fate of the unaffected sheep which showed positive staining, and other sheep in the trial, is yet to be reported. However the application of this technique as a risk management measure is restricted as the amount of lymphoid tissue in the nictitating membrane decreases with age (Ron Rogers *pers comm*).

Conclusion

The use of an immunohistochemical test on selected tissues including brain, brain stem, spinal cord, spleen, mesenteric lymph nodes and palatine tonsils obtained at autopsy from donors of semen or embryos would considerably reduce the probability of collecting genetic material from susceptible sheep incubating scrapie.

4.6 IDENTIFICIATION OF DONORS

There is a risk that imported genetic material may not have been collected from the selected donors which met conditions. This could be the result of intentional non-compliance or mistakes made during the collection, handling and storage prior to export. This risk can be managed by requiring identification of donors and by including requirements which enable the confident matching of imported genetic material with the identified donor.

Donors may be individually identified by ear tags or transponders (microchips). Donors can be matched with imported genetic material by characterising the DNA of each donor and matching this with either the imported semen or progeny from the imported embryos. Individual DNA characteristics can be determined from a blood sample, a semen sample or even a hair follicle.

Conclusion

The use of accurate identification systems to match genetic material with donors would reduce the probability of substitution and hence the probability of importing genetic material from donor sheep incubating scrapie.

4.7 HANDLING, PROCESSING AND STORAGE OF SEMEN AND EMBRYOS

The application of appropriate sanitary precautions considerably reduces the risks of semen and embryos carrying conventional pathogens. Sanitary procedures for international trade in genetic material are laid out in the OIE *Code* which includes recommended methods for testing donors as well as the choice and use of equipment and media. Careful and disciplined use of equipment and media is of particular significance with scrapie and other TSEs. To prevent transmission between donors extreme care must be taken with instruments used during the collection and transfer of embryos and semen. To treat instruments such as laparoscopes and electro ejaculators in a manner known to remove scrapie infective agent could be difficult. A recommended treatment for instruments used on patients with CJD is for the instruments to be immersed in 1 Normal NaOH for one hour, then cleaned before being autoclaved at 134 degrees C for 1 hour (World Health Organisation, 1998).

In *Code* Appendix 4.2.3.3. under *Media* it is recommended that *any biological product of animal origin …should be free of living micro-organisms.* It is doubtful if SIA could be classified as a *micro-organism*. Materials of animal origin used in semen storage and embryo production and transfer should be sourced only from scrapie and BSE free countries and subjected to quality control methods in accordance with Chapter 10 of the Manual of the International Embryo Transfer Society (IETS) 3rd edition, 1998.

Scientists involved in scrapie research agree that the application of appropriate sanitary procedures during the collection, handling, storage and transfer of semen and embryos considerably reduces the risk of transmission.

Conclusion

The collection, handling and storage of ovine semen and embryos in accordance with OIE Code recommendations would reduce the risk of transmission of pathogens, including SIA. Additional measures, known to destroy SIA, would further reduce the risk of transmitting SIA on instruments used during collection and transfer.

5 RISK MANAGEMENT - CAPRINE SEMEN AND EMBRYOS

Scrapie is a rare disease of goats. The risk of importing SIA in caprine genetic material is far less than that associated with ovine genetic material. No single measure or subgroup of measures would achieve Australia's appropriate level of protection (ALOP). However, on the basis that the new measures, in combination, meet the ALOP and are significantly less trade restrictive than the current conditions (based on SFAP) AQIS proposes to apply them to the importation of goat embryos from Canada, the USA and Member States of the EU.

Selection of embryo and semen donors based on the history of the herd of origin and the age of the animal reduces the probability of using a scrapie infected donor. For the reasons given in this section, these measures, even when combined with handling, processing and storage of semen and embryos in accordance with the relevant OIE Animal Health *Code* requirements do not reduce the risk to achieve Australia's appropriate level of protection (ALOP). The limited information available indicates that testing donor tissues after autopsy, alone or in combination with *Code* recommended standards for semen and embryos, can also reduce the probability of using a scrapie infected donor. However, the level of risk reduction is uncertain. As is the case with sheep, not enough is known of the time course of the SIA tissue distribution to be confident that this form of testing can detect infected animals at all stages of the disease.

Each of the above measures can reduce the probability of collecting infected embryos or semen but no single measure or subgroup of measures would achieve Australia's ALOP. However, on the basis that the new measures, in combination, meet the ALOP and are significantly less trade restrictive than the current conditions (based on SFAP) AQIS proposes to apply them to the importation of goat embryos from Canada, the USA and Member States of the EU.

5.1 HISTORY OF SCRAPIE IN THE FLOCK OF ORIGIN

Naturally acquired scrapie in goats is normally reported in goats that have been in close contact with infected sheep (Toumazos and Alley 1989; Capucchio *et al* 1998). Other authors have speculated that contaminated feed may have been the source of SIA (Andrews *et al* 1992). After studying the concentrations of SIA in various tissues of naturally infected goats, Hadlow *et al* 1980 concluded that scrapie could be maintained in a herd of goats living apart from infected sheep. Lateral transmission has been reported in goat herds but there are no reports of maternal transmission (Wood *et al* 1992).

Conclusion

The probability of collecting genetic material from infected goats would be considerably reduced if the donors came from a flock with no history of scrapie and which had not been in contact with sheep.

5.2 SUSCEPTIBILITY OF GENOTYPE

Analysis of the caprine PrP gene has revealed several different alleles. Four PrP protein variants were found three of which were goat specific with single amino acid changes at codons 142, 143 and 240. Dimorphism at codon 142 appeared to be associated with different incubation periods in

goats experimentally infected with BSE and two strains of scrapie (Goldman *et al* 1996). Earlier work indicated that nearly all goats were susceptible to scrapie and that the incubation period varied between 12 and 22 months (Wrathall 1997). A novel PrP gene allele is associated with a longer incubation period (to 968 days) post-inoculation (Goldman *et al* 1998).

5.3 AGE OF DONOR

There is little published information on the influence of age on the development of scrapie in goats. However during one outbreak of natural scrapie goats died between 2 and 7 years of age (median of 3 years) with 85 percent dying between 2 and 4 years (Wood *et al* 1992).

Conclusion

The probability of collecting genetic material from infected donors would be reduced if the donors were over 5 years of age at the time of collection.

5.4 TESTING OF DONORS

Virological and neurohistological findings in 3 dairy goats with natural scrapie were essentially similar to those in infected sheep. SIA was widespread in non-neural sites particularly in lymphatic tissue.

Conclusion

The use of immunohistochemical tests for the detection of scrapie PrP in selected tissue of donor goats at autopsy, would reduce the probability of collecting genetic material from goats incubating scrapie.

5.5 HANDLING, PROCESSING AND STORAGE OF SEMEN AND EMBRYOS

The same comments apply to caprine embryos and semen as apply to ovine embryos and semen.

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