DISEASE RISK ASSESSMENT

This import risk analysis was conducted and documented by a private consultant working on behalf of a would-be importer. It is not an official MAF Biosecurity Authority risk analysis.

Nevertheless, this risk analysis has been subjected to MAF's internal scientific review process and to an external expert review. The risk analyst has addressed all the points raised by MAF and the external reviewers.

MAF considers this risk analysis to be technically sound and sufficiently robust for an Import Health Standard to be developed from.

THE USE IN NEW ZEALAND OF IMPORTED SEMEN DERIVED FROM AN ARGALI (*Ovis ammon polii*) SHEEP

PREPARED FOR DIVERSE AGRICULTURAL HOLDINGS LTD
BY
PETER O'HARA BVSc(Hons), PhD.

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Summary

This assessment of the risks associated with the use in New Zealand of semen from an Argali (*Ovis ammon polii*) ram forms part of an application to MAF for the promulgation of an import health standard and permit for its use. The risks attached to the inoculation into goats of homogenized mesenteric lymph node removed surgically from the ram are also assessed. This inoculation could be undertaken to test for the existence of scrapie prions in the ram.

The ram was born in a Chinese zoo and was imported with two Argali ewes and four sheep of another breed into the Singapore zoo in October 1998. The two Argali ewes died within 6 months of their arrival. The ram had been in the zoo for 21 months prior to the first semen collection and remains resident there. The semen and other samples were collected at the zoo.

While the health history of the ram while in Singapore can be viewed with some confidence, there are sufficient uncertainties about its history of disease exposure prior to entering Singapore that a cautious approach with respect to diseases having long incubation periods is indicated.

The approach to the assessment of disease risks was to consider that any introduction of the diseases discussed below carried unacceptable animal health and economic consequences and therefore the probability of the risks should be negligible.

On the grounds that Singapore is recognized by the Office International des Epizooties (OIE) as being free of foot-and-mouth disease and rinderpest and, with the exception of bluetongue, the other acute and sub-acute diseases on OIE's List A have distributions that do not include South East Asia, the ram presents a negligible risk of infection. Some uncertainty around bluetongue exists because of its occurrence in the region, the distribution of competent insect vectors is unknown and Singapore does not have an active surveillance programme. Testing the ram's sera for bluetongue is recommended.

Of the other diseases considered, the probability of the ram's exposure to three diseases, scrapie, pulmonary adenomatosis and maedi-visna, can not be determined with certainty. All three diseases are characterised by prolonged incubation periods that would include the period of residence of the ram in China. The status of these diseases in China is not known. For these reasons, it is recommended that the ram's sera is tested for maedi-visna, the mesenteric lymph node is inoculated intra-cerebrally into goats that are then observed while in MAF-authorised and supervised containment for a period of three years and that the inseminated ewes and their progeny are observed while in containment for a period of three years. Because it is suspected that Q fever can be transmitted via bull semen, it is recommended that the ram be tested serologically for Q fever.

The inoculation of the mesenteric lymph node into goats does not present risks that are not managed through the protocol described above.

1. Introduction

This disease risk assessment has been prepared as part of an application to MAF to issue a permit and import health standard that will allow the semen described below to be used to inseminate New Zealand ewes to create cross-bred Argali sheep.

The semen, blood and tissue samples listed in the next section have been collected from an Argali (*Ovis ammon polii)* ram and were imported into New Zealand under MAF Permit Number 2000010925 on 25 January 2001 (Documents I and II). A condition of the permit was that the material was held in storage in a transitional facility until such time as further import health standards and permits were issued that would enable the semen to be used for reproductive purposes. The samples are currently held in secure storage in a MAF-registered transitional facility.

2. Imported material

SAMPLE	DATE COLLECTED
Mesenteric lymph node	7.4.00
Serum	8.3.00
Serum	7.5.00
Serum	25.10.00
Plasma	25.10.00
Whole blood	25.10.00
Semen straws	13.7.00
Semen straws	14.8.00
Semen straws	14.9.00

The semen was obtained by electro-ejaculation and was diluted in a TRIS-based diluent containing 10% egg yolk, 7% glycerol, plus penicillin and streptomycin sulphate in final concentrations of 1 million IU per 100 ml and 1mg per 100 ml respectively. The egg yolk was the only product of animal origin in the diluent. The eggs were purchased in a Singapore supermarket and are of Singapore origin.

All of the above samples are held frozen in liquid nitrogen.

The intended use of the materials listed above is as follows:

Semen:	inseminate New Zealand bred ewes to create cross-bred sheep;
Serum, plasma and whole blood:	materials for any laboratory testing that may be required as a condition of an import health standard;
Mesenteric lymph node:	inoculation of goats as part a scrapie freedom assurance programme (SFAP) should this be required as a condition of an import health standard

3. History of the Donor Animal

The donor ram is identified by a microchip 00-01BD-4FBB (ISIS accession # G6154).

The Argali ram was imported into Singapore with two Argali ewes and four Bharal sheep from a Chinese zoo on 11.10.98 and has been resident in Singapore since that time. The two ewes died within 6 months of importation as the result of clostridial infections of wounds (diagnosis made by the zoo veterinarian).

The ram was born in captivity. In the course of three visits in the period of two years prior to the export of the ram to Singapore, the importer has established that the Chinese zoo has held a breeding flock of Argali sheep for at least 15 years. It is not known whether introductions were made from the natural range or other sources during earlier years but zoo staff stated that no introductions were made in the two years prior to the export of the ram to Singapore. Because of the small number of animals involved, the importer was able to confirm identifications of individual animals and determine that this statement was correct.

Ma Guiping, a Chinese Government Veterinary Officer certified (Document III) in September 1998 that

1. the ram was derived from premises that had been free from foot-and-mouth disease, rinderpest, contagious bovine pleuropneumonia, peste des petits ruminants, contagious caprine pleuropneumonia and bluetongue for at least 12 months prior to export;
2. the ram had been kept in captivity in the premises of origin for at least 6 months and in a quarantine station for at least 40 days prior to export;
3. the premises of origin was in an area in which infectious and contagious diseases were under control and where no case of trypanosomiasis, anaplasmosis, Johne's disease, rabies or brucellosis had occurred in the 3 months prior to export;
4. the ram had not received any vaccine in the 30 days prior to export;
5. the ram was clinically healthy and free of clinical signs of infectious disease at the time of export; and
6. he was protected from insect vectors during pre-export quarantine and transportation to the place of shipment.

The ram, the ewes and the four Bharal sheep were tested for bluetongue two months after arrival in Singapore using an ELISA test at a dilution of 1:10. All animals gave negative results (Document IV).

The ram has been held in the same facility since it arrived in Singapore and has been in contact with other ruminant species held in that facility.

Dr Eugene Chan, a Singapore Government veterinary officer certified (Document V) on 18 January 2001 that the ram:

1. was 6 years old at the time of the collection of the samples listed above;
2. has been continually resident in the Singapore Zoological Gardens for the 12 months prior to collection of the samples ;
3. remained healthy throughout the collection period as did all in-contact animals; and
4. none of the ruminants at the zoo have been under any official quarantine restrictions in the 6 months preceding the collections listed above.

4. Risk Analysis Methodology

The methodology used in this disease risk assessment follows the procedures outlined in the *International Animal Health Code* of the Office International des Epizooties (OIE) and includes:

- disease hazard identification;
- release assessment (the biological pathways by which an infectious agent might be transmitted through the use of the semen);
- exposure assessment (the biological pathways by which susceptible New Zealand animals might be exposed to any infectious agent derived from the semen); and
- consequence assessment (the health and economic consequences of introducing an unwanted infectious agent through the use of the semen).

The risk assessment has concentrated on two factors:

1. evidence of the state of health of the donor ram at the time of collection of the semen and possible exposure of the ram to infectious agents that are listed below as hazards to New Zealand; and
2. release assessment: evidence that the semen can act as a vehicle for the transmission of infectious agents that the donor ram could be infected with.

In making an assessment of the magnitude of any risks, greater weight has been placed on the state of the health of the donor ram than on whether or not semen is a recognized vehicle for transmission of infection. The focus was on the probability component of the risk because it was taken as granted that the consequences of the introduction of an exotic organism into New Zealand carried high health and economic costs. Assessments of the state of health of the ram in respect of some of the disease hazards can be made with confidence while for others, uncertainty exists. This is discussed in more detail below.

The risk management standard used in this analysis is that the risk of the release of any unwanted organism discussed below and exposure of susceptible indigenous animals should be negligible. Risk management safeguards to achieve this standard are recommended for those disease hazards that would carry a level of risk in the un-modified state higher than would be acceptable to New Zealand.

5. Disease Hazards and Risks

The diseases of sheep that are hazards and are potential risks considered in this analysis are:

1. the acute infectious diseases of sheep that are included in "List A" by the OIE. They are:
   - foot and mouth disease (FMD)
   - vesicular stomatitis
   - rinderpest
   - peste des petits ruminants
   - Rift Valley fever
   - bluetongue
   - sheep and goat pox.

None of these diseases have ever occurred in New Zealand

2. Other diseases exotic to New Zealand including those that are associated with reproduction and/or might be transmitted via semen are:
   - contagious agalactia
   - enzootic abortion of ewes
   - ovine pulmonary adenomatosis
   - salmonellosis (*Salmonella abortus-ovis*)
   - scrapie
   - maedi-visna
   - brucellosis (*Brucella melitensis*)

3. Diseases that OIE recommends be considered in semen donors but that are endemic in New Zealand are:
   - paratuberculosis
   - ovine epididymitis
   - caseous lymphadenitis.

The risks assessed relate to the use of the semen to inseminate New Zealand ewes and to the inoculation of goats with mesenteric lymph node as part of a SFAP.

6. Risk Assessment

6.1 The OIE List A Diseases

The diseases of sheep designated as the OIE List A diseases above are all virus diseases. In assessing the risk probability that the donor ram was infected at the time of collection of the semen, three factors are particularly important:

1. exposure of the ram to a source of infection;
2. the incubation periods of the diseases; and
3. any clinical or laboratory testing evidence.

6.1.1 Health Status of Singapore

Notwithstanding the ram's origin in China, it had been resident in Singapore for 18 months prior to the collection of the first serum samples and 22 months prior to the collection of the first semen. The List A diseases with the exception of bluetongue have incubation periods of 14 to 30 days[a] . The maximum infective period for bluetongue is considered to be 60 days[a] . Thus, in respect of the acute and sub-acute infectious diseases of sheep, the ram should be regarded as a Singaporean sheep.

At the time of the importation of the samples listed above, Singapore was certified free of foot and mouth disease, vesicular stomatitis, rinderpest, peste des petits ruminants, sheep and goat pox, bluetongue and Rift Valley fever. This certification is consistent with the health status of Singapore as reported to and by OIE. There is no reason to question the veracity of this certificate. OIE recognizes Singapore as being free of FMD and rinderpest(see below).

Singapore has a small animal population. The following figures are estimates included in FAOSTAT Agricultural Data,2000:

Cattle	200
Buffalo	20
Goats	300
Pigs	190 000

a. OIE. International Animal Health Code, 10th Edition, 2001.

6.1.2 Risk Assessment of the Specific Diseases

6 1.2.1 Foot and Mouth Disease

FMD is an acute, highly contagious disease of cloven hoofed animals caused by an aphthovirus (family Picornaviridae). There are seven distinct serogroups of the virus and within some of the groups, a number of topotypes. Cross reactivity between topotypes is variable.

Spread is by aerosol or ingestion[1]. Incubation periods are usually short (less than 14 days[2]). Viraemia occurs during the incubation period and extends into the clinical phase. Clinical signs are characterized by fever and vesicles in and around the oral cavity and on the coronary bands and interdigital clefts of the feet. The symptoms in sheep can be mild and difficult to detect.

Persistent infections can occur in cattle and African buffalo[3] but are not recorded in other species.

Although FMD is endemic or sporadically epidemic in parts of South East Asia, Singapore is recognized by OIE as free (OIE International Committee Resolution XVII. May 2001). FMD was last recorded in 1935.

Release assessment: Based on research in cattle[4,5], virus is likely to be present in semen during the incubation and clinical phases of the disease. Levels of virus in the semen are sufficient to infect other cattle by insemination with frozen semen. Although persistent infections in cattle have been demonstrated, infection becomes confined to the pharynx and levels of virus are not sufficient to cause infection in in-contact animals[6].

Conclusions: Given Singapore's freedom from FMD and the length of stay of the ram, it is considered that there is no risk of the ram's tissues or semen containing FMD virus.

1. Sanson, RL: the epidemiology of foot and mouth disease. Implications for New Zealand. NZ vet J42, 41-53.1994.
2. OIE International Animal Health Code,10th Edition, 2001. Chapter 2.1.1.
3. Thompson, GR: The role of carrier animals in the transmission of foot and mouth disease. Proceedings of the OIE General Session, May 1996, pp87-103.
4. Cottral, GE; Gailiunas,P; Cox, BF: Foot and mouth disease in semen of bulls and its transmission by artificial insemination. Arch fur die gesamte Virus Forschung 23, 362-77,1968.
5. Sellars, RF; Burrows, R; Mann, JA; Dawe,P: Recovery of virus from bulls affected with foot and mouth disease. Vet Rec 83,303,1968.
6. Sellers RF: Quantitative aspects of the spread of foot and mouth disease. Vet Bulletin 41, 431-9,1971.

6.1.2.2 Vesicular Stomatitis

Vesicular stomatitis is a disease caused by a vesiculovirus (family Rhabdoviridae) characterised by vesicle formation that is indistinguishable from FMD in cattle and pigs[1] . It is primarily an infection of cattle, horses and pigs but sheep and other species including man can be affected.

Infection occurs via trans-cutaneous and trans-mucosal routes. Epidemics are seasonal and associated with the presence of virus in biting arthropods indicating probable insect borne transmission[2]. The incubation period is up to 21 days[3].

The virus is confined to the Americas[1] and has never been reported in Singapore or South East Asia[4].

Release Assessment: Based on the distribution of the disease, vesicular stomatitis is not a hazard in this ram's semen or tissues.

1. OIE. Manual of Standards for Diagnostic Tests, 4th Edition, 2000.Chapter 2.1.2.
2. Letchworth GJ, Rodriguez LL, Barrera J, Del C. Vesicular stomatitis. Vet J 157,239-60,1999
3. OIE. International Animal Health Code, 10th Edition, 2001. Chapter 2.1.2.
4. OIE. HANDISTATUS II Annual Animal Health Status, 2000

6.1.2.3 Rinderpest

Rinderpest is an acute fatal disease caused by a morbillivirus (family Paramyxoviridae). It is primarily a disease of cattle, buffalo and yak but sheep and other ungulates can be affected[1]. The disease is characterized by fever and erosions of the mucous membranes of the mouth, a nasal discharge and diarrhea and dysentery. The incubation period is up to 21 days[2].

The virus is present in two forms in East Africa and a third form is endemic in Pakistan[1].

Rinderpest last occurred in Singapore in 1930 and OIE recognizes Singapore as a rinderpest free country (OIE International Committee Resolution XVI, May 2001).

Release Assessment: Given Singapore's internationally recognized freedom, rinderpest is not a hazard in this ram's semen or tissues.

1. OIE. Manual of Standards for Diagnostic Test and Vaccines, 4th Edition, 2000. Chapter 2.1.4.
2. OIE. International Animal Health Code, 10th Edition, 2001. Chapter 2.1.4.

6.1.2.4Peste des petits ruminants

Peste des petits ruminants is caused by a morbillivirus (family Paramyxoviridae) and closely resembles rinderpest. It affect small ruminants especially goats and occasionally wild animals[1].

It occurs in sub-Saharan Africa, the Middle east and south west Asia. It has never been reported in Singapore[2].

Release Assessment: The known distribution of the disease and Singapore's freedom mean that peste des petits ruminants is not a hazard in this ram or its tissues.

1. LeFevre PC, Diallo A. Peste des petits ruminants. Rev Sci Tech Off int Epiz, 9, 951-65, 1990.
2. OIE. HANDISTATUS II Annual Animal Health Status,2000

6.1.2.5 Rift Valley fever

Rift Valley fever is a mosquito borne virus disease that affects a range of animals and man but is most severe in sheep, goats and cattle. It is caused by *Phlebovirus* (family Bunyaviridae). The distribution of the virus varies in accordance with the distribution of the mosquitoes (a number of species are implicated) which, in turn, is dependent on seasonal rainfall patterns[1]. Incubation is up to 30 days[2].

While the mosquitos of South east Asia are potential vectors of the virus, the disease has not been reported outside Africa and the Middle East. Its occurrence in North Africa and the Middle East is episodic and related to mosquito distribution following rain[1].

Release Assessment: The known distribution of the disease and the stated freedom of Singapore mean that the ram or its tissues or semen present no risk.

1. Swanepoel R. Rift Valley fever. In *Infectious Diseases of Livestock with Special Reference to South Africa*, Vol 1, (ed Coetzer JAW, Thompson GR, Tustin RC), Oxford University Press, Cape Town, 1994, pp688-718.
2. OIE. International Animal Health Code, 10th Edition, 2001. Chapter 2.1.8.

6.1.2.6 Bluetongue

Bluetongue is caused by an orbivirus (family Reoviridae) and is spread by species of the midge *Culicoides*. A wide range of domestic and wild ruminants are susceptible to infection which results in overt disease in some species such as sheep or inapparent infections such as in cattle and the majority of other species[1,2]. The 8 Australian serotypes are mildly to moderately pathogenic to

sheep in experimental infections but infection in the field is unusual and clinical disease is not seen[3].

The maximum infective period is 60 days[4].

Clinical disease in sheep is characterized by fever and hyperaemia, oedema, congestion, haemorrhages and erosion of the mucous membranes of the mouth and nose. These lesions may extend to the wool-less areas of skin of the body and the feet, the latter resulting in lameness.

The virus is widely distributed throughout the world and can be considered to be a risk wherever species of *Culicoides* exist. Seventeen species of *Culicoides* world wide are known to be competent vectors[3]. The disease or infection has never been reported in Singapore[5] but this is based on the absence of clinical disease and there is no active surveillance system in place

Release Assessment: Experimental studies of infection in bulls has demonstrated that virus can be present in semen during viraemia but does not persist after that time[6]. Earlier research that suggested long term persistent shedding in semen has been discredited. Infected semen is capable of infecting inseminated heifers[7]. If it is assumed that sheep behave similarly, the risk of the ram's semen containing virus would depend on the ram being viraemic at the time of collection.

The risk of transmission of bluetongue to New Zealand goats used for inoculation of mesenteric lymph node in a SFAP is also related to the presence or absence of viraemia.

While the ram and its cohorts were sero-negative two months after their arrival in Singapore and Singapore considers itself to be free of infection, the distribution of infection in the region and the absence of a surveillance system for bluetongue in Singapore suggests some uncertainty as to the true status of the ram.

Midges of the genus *Culicoides* are not found in New Zealand making it probable that any infection is self-limiting.

Proposed risk management: It is recommended that the ram is tested serologically for the presence of bluetongue antibodies. Since serial serum samples are available, if a rising titre is demonstrated, it is recommended that the mesenteric lymph node and semen not be used. Because sheep are considered to be more susceptible than most other species, the absence of clinical disease in the ram can be taken to suggest a low probability of infection.

1. Verwoerd DW, Erasmus BJ. Bluetongue. In *Infectious Diseases of Livestock with Special Reference to South Africa* Vol 1 (ed. Coetzer JAW, Thompson GR, Tustin RC), Oxford University Press Cape Town, pp 443-59, 1994
2. OIE. Manual of Standards for Diagnostic Tests and Vaccines, 4th Edition, 2000. Chapter 2.1.9.
3. Geering WA,Forman AJ, Nunn MW. Exotic Diseases of Animals, Australian Government Publishing Services, Canberra, 1995, pp61-70.
4. OIE. International Animal Health Code,10th Edition, 2001. Chapter 2.1.9.

5. OIE. HANDISTATUS II Annual Animal Health Status, 2000.
6. Bowen RA, Howard TH, Entwhistle KW,Pickett BW. Seminal shedding of bluetongue virus in experimentally infected mature bulls. Am J vet Res, 44, 2268-70, 1983.
7. Bowen RA, Howard TH. Transmission of bluetongue virus by intrauterine inoculation or insemination of virus containing bovine semen. Am J vet Res, 45, 1386-8,1984.

6.1.2.7 Sheep and goat pox

Sheep and goat pox are caused by strains of the capripox virus. The clinical disease is characterised by typical pox lesions, fever and by lesions in the lungs and elsewhere. Death is a common outcome in lambs and occurs in 5 to 50% of adults[1,2]. The virus is distinct from parapox virus that causes contagious pustular dermatitis (scabby mouth).

Sheep and goat pox is endemic in Africa, the Middle East and the Asian sub-continent. It has never been reported in Singapore[3].

Release Assessment: The known geographical distribution of the disease and the freedom of Singapore indicate that the ram presents no risk of this disease. A chronic carrier state does not occur and clinical disease is a reasonably reliable indicator of the existence of the disease.

1. OIE. Manual of Standards for Diagnostic Tests and Vaccines, 4th Edition, 2000. chapter 2.1.10.
2. Coetzer JAW, Thomson GR, Tustin RC (eds). Infectious Diseases of Livestock. Oxford University Press, Capetown, 1994. p614.
3. OIE. HANDISTATUS II Annual Animal Health Status, 2000.

6.2 Other diseases including those that may be transmitted through semen

The OIE International Animal Health Code[1] recommends *inter alia* that sheep semen donors be derived from flocks that are free of

- contagious agalactia (for at least 6 months);
- caseous lymphadenitis and ovine epididymitis (for at least 12 months);
- paratuberculosis (for at least two years); and
- scrapie, pulmonary adenomatosis and maedi-visna (for at least three years).

The diseases in this group are more chronic in character than the List A diseases discussed above, have longer incubation periods and may exist in an animal in a sub-clinical but infectious form for long periods. The period of freedom of flocks from infection recommended by OIE reflects these characteristics.

Singapore claims freedom from these diseases[2]. The status of China in respect of these diseases is not reported to OIE[2] and can not be determined with any certainty. In assessing the risk of the ram being infected with any of these diseases, the

absence of any satisfactory history of exposure or non-exposure in China and dependence of Singapore on the absence of clinical disease as the basis for its declaration of status leads to a conclusion that exposure and infection of the ram is possible. Given the age of the ram (6 years) and its freedom from clinical signs, the probability of infection is low but can not be determined with any real precision.

1. OIE. International Animal Health Code,2000. Appendix 3.2.2.
2. OIE. HANDISTATUS II Annual Animal Health Status, 2000.

6.2.1 Contagious agalactia

Contagious agalactia is a disease of sheep and goats in which a number of species of *Mycoplasma* (*M. capricolum* subsp *capricolum, M mycoides* subsp *mycoides, M. agalactiae* and others) have been implicated[1,2].

The course of the disease is one to several months and the clinical signs include mastitis, ophthalmitis, arthritis and abortion. The disease is often more severe in pregnant sheep[1]. Lambs may be stillborn or born weak and die shortly after birth. Pneumonia may also be a feature.

Infection occurs through exposure to infected sheep and to foetal membranes and discharges following abortion. Infection of rams has not been implicated in transmission of the disease.

Contagious agalactia is endemic in Mediterranean countries notably Spain and has been reported from the Canary Islands and Ethiopia. Singapore claims freedom on the basis of non-occurrence of clinical disease[3].

Release assessment: The ram has not lived in a flock of its own kind for most of the time it has been in Singapore, has not been sexually active and has had no known exposure to infection for at least 15 months.
Exposure assessment: Venereal transmission has not been implicated in the epidemiology of the disease.

1. Radostits OM,Gay CC,Blood DC, Hinchcliff KW. Veterinary Medicine, 9th Edition, WB Saunders, London, 2000, p1005.
2. OIE. Manual of Standards for Diagnostic Tests and Vaccines, 4th Edition, 2000. Chapter 2.4.3.
3. OIE. HANDISTATUS II Annual Animal Health Status 2000.

6.2.2 Enzootic abortion

Enzootic abortion is a disease of sheep and goats caused by *Chlamydia psittaci* Biotype1/Serotype1 (now called *Chlamydophila abortus*[2]).

Infection is usually introduced into a flock through the introduction of latently infected females that subsequently abort at the end of their next pregnancy. Once

established in a flock, lateral spread through ingestion is the principal means of spread. The environment of a flock becomes contaminated by infected foetal membranes and discharges[1]. Infection can persist in the female reproductive tract[1].

The clinical disease is characterized by abortion, stillbirths and the birth of weak lambs.

Enzootic abortion is one of the main causes of abortion in sheep in the UK and is also prevalent in the USA. It has not been reported in Singapore[3].

Release assessment: The ram has not lived in a flock of its own kind for most of the time it has been in Singapore, has not been sexually active and has had no known exposure to infection for at least 15 months.
Exposure assessment: Venereal transmission has not been implicated in the epidemiology of the disease. Enzootic abortion is primarily a disease of the female and transmitted by females. It is not considered significant in artificial insemination. However, OIE[4] recommends that semen donors be tested serologically for *Chlamydia* 2 to 3 weeks post collection and the semen cultured for *Chlamydia psittaci*.

1. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary Medicine. 9th Edition, WB Saunders, London, 2000, p1259.
2. OIE. Manual of Standards for Diagnostic Tests and Vaccines, 4th Edition, 2000. Chapter 2.4.7.
3. OIE. HANDISTATUS II Annual Animal Health Status, 2000.
4. OIE. International Animal Health Code , 10th Edition , 2001. Chapter 2.4.7.

6.2.3 Salmonella abortus –ovis

Abortion caused by *Salmonella abortus-ovis* is uncommon[1]. It is noteworthy that other Salmonellas such as *S. dublin, S. typhimurium* and *S. brandenburg* may be associated with abortion.

Infection is spread by carrier animals and may persist in such animals for up to 6 months. The bacterium is excreted in faeces and the vaginal discharges of aborted sheep. While rams can become infected, the evidence is against spread by coitus[1]. Experimentally, infection of rams by the preputial route may lead to shedding of the bacterium in semen for up to 15 days.

Clinical signs are abortion in late pregnancy, some mortality in ewes and pneumonia in lambs up to 2 weeks old.

Salmonella abortus-ovis has not been reported in Singapore[2].

Release assessment: The ram has not lived in a flock of its own kind for most of the time it has been in Singapore, has not been sexually active and has had no known exposure to infection for at least 15 months.
Exposure assessment: Venereal transmission has not been implicated in the epidemiology of the disease.

1. Radostits OM, Gay CC. Blood DC, Hinchcliff KW. Veterinary Medicine, 9th Edition. WB Saunders, London, 2000. p828.
2. OIE. HANDISTATUS II Annual Animal Health Status, 2000.

6.2.4 Pulmonary adenomatosis

Pulmonary adenomatosis of sheep and goats is a virus induced bronchoalveolar adenocarcinoma. The causative virus is believed to be a retrovirus with strong homology to the B and D prototype oncoviruses. Because the virus has not been isolated in the laboratory, final confirmation of its role has not been obtained. A herpesvirus was implicated at one time but it is now believed to have no role in the disease[1].

The disease is characterized by a long incubation (1-3 years) and a prolonged clinical course. The onset of the clinical disease is insidious and may not be noticed. While the prevalence of clinical disease is usually less than 5%, post-mortem evidence shows that the prevalence of lung tumours can be much higher[1].

The method of transmission is believed to be by inhalation of infected droplets shed by affected sheep. Experimentally, it has been transmitted by suspensions of lung material[1].

The disease has been reported from the UK, Europe, Africa, the Middle East, India, China, North America and some South American countries[2]. It has not been reported in Singapore[3].

No tests are available for identification of infected animals or flocks[1].

Release Assessment: While there is no specific evidence of transmission by semen, the unusual nature of the disease (oncogenic virus), the lack of a test for infection and the long incubation period dictate a cautious approach. Observation of progeny in long term quarantine and post-mortem of any progeny dying in quarantine or sentinel goats dying during the quarantine period or destroyed at the end of the period would be an appropriate procedures.

1. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary Medicine, 9th Edition, WB Saunders, London, 2000. p1190-91.
2. Geering WA, Forman AJ, Nunn MW. Exotic Diseases of Animals, Australian Government Publishing Service, Canberra, 1995. pp199-202.
3. OIE. HANDISTATUS II Annual Animal Health Status,2000.

6.2.5 Maedi-visna

The disease is commonly referred to by its Icelandic names (maedi = pneumonia, visna = nervous disease) but is also known as ovine progressive pneumonia. It is caused by a lentivirus one of a group of non-oncogenic retroviruses. The maedi virus is closely related by nucleotide homology and serologically to the caprine arthritis-encephalitis virus[1].

Infection can persist in the face of an immune response and one explanation offered for this is that the virus nucleic acid can become associated with host cellular DNA and thus be protected from antibodies[2]. The virus also demonstrates antigenic drift and considerable heterogeneity which may also be protective of the virus[2].

Infection is transmitted by droplet inhalation, ingestion of colostrums and milk of infected ewes and by contact when sheep are held in confinement. The severity of the outbreak in Iceland was attributed to the long period of housing of the sheep during the winter months[1]. Virus has been demonstrated in the semen of infected rams that have leukospermia[1].

The incubation period is usually greater than 2 years although it may be shorter for visna[2]. The clinical disease is manifest as a progressive pneumonia, encephalitis, mastitis and arthritis[1].

The disease has been reported from all of the major sheep producing countries except New Zealand, Australia, Finland and Iceland (where it has been eradicated). The prevalence in the USA can be quite high. In one study, 26% of individual sheep bloods submitted to diagnostic laboratories were seropositive and 48% of the flocks from which the samples were taken had at least one seropositive sheep[1]. The disease has not been reported in Singapore[3].

Satisfactory serological tests are available although, as with all serological tests, infected individuals can be negative[4].

Release assessment: The long incubation period of the disease and the uncertainty surrounding the history of the ram in China mean that the possibility of infection can not be ruled out. The age of the ram suggests that the probability of him being sub-clinically infected is low. Because Singapore has only a small goat population and very few sheep, the probability that its declared status of freedom is correct is high.
Exposure assessment: Establishment of infection in New Zealand would require the ewes inseminated with the ram's semen to become infected and for them to transmit infection to their offspring. Even though the probability of this occurring is low, prudence suggests that a controlled situation to test this eventuality is required.

Proposed risk management: It is recommended that the ram's sera be tested serologically (agar gel diffusion or ELISA). If antibodies are demonstrated, the titres should be assessed. The use of the semen should not be ruled out if stable levels of antibodies are found and consideration should be given to serological surveillance of the offspring while in long term quarantine (see below). Sheep or sentinel goats that die during the quarantine period should be subjected to post-mortem examination.

1. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary Medicine, 9th Edition, WB Saunders, London,2000. pp1186-89.
2. Geering WA, Forman AJ, Nunn MW. Exotic Diseases of Animals, Australian Government Publishing Service, Canberra, 1995. pp163-67.
3. OIE. HANDISTATUS II Annual Animal Health Status, 2000.
4. OIE. Manual of Standards for Diagnostic Tests and Vaccines, 4th Edition, 2000. Chapter 2.4.4/5.

6.2.6 Scrapie

Scrapie is now regarded as an infectious disease caused by transmission of prions, infectious proteins that are capable if inducing transformation of normal host cell membrane glycoproteins (PrP^{C}) to an abnormal fibrillar form (PrP^{sC}) resembling themselves. The disease also has a genetic basis that determines susceptibility, the length of the incubation period (*sip* gene in sheep, *sinc* gene in mice) and the nature of the clinical disease and the neuropathology[1]. Fifteen strains of the infectious agent have been characterized through studies in mice, sheep and goats.

The disease has a long incubation period. Clinical disease can occur as early as 18 months post infection but most cases are 2.5 to 4.5 years old. Incubation periods in goats are shorter than for sheep[1].

The classical clinical signs of scrapie are a slowly progressive debilitation of neuromuscular function and intense pruritis. However, there are considerable variations in the expression of symptoms. The characteristic and diagnostic lesions in the central nervous system are neuronal vacuolation, spongiform degeneration of the neuropil and the accumulation of amyloid like fibrils.

While the spread of the disease is attributed largely to environmental contamination by infected foetal membranes and fluids at parturition, other methods of spread have been suggested. Infective material has not been demonstrated in testes or semen but vertical transmission through semen can not be ruled out unlike BSE[2].

Release assessment: The uncertain history of this ram, the many uncertainties about the epidemiology of scrapie including the inability to rule out transmission via semen dictate a precautionary approach.

1. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary Medicine, 9th Edition, WB Saunders, London, 2000. pp1228-33.
2. OIE. International Animal Health Code, 10th Edition,2001. Chapter 2.3.13.

6.2.7 Q fever

Q fever is caused by *Coxiella burnetii* which cycles in cattle, sheep, goats and a wide range of wild animals and their ectoparasites[1,2]. Latent infections occur in the udder and uterus of ruminants, these being activated by pregnancy and the organism is shed at parturition. Q fever is an important zoonosis.

C. burnetii has been demonstrated in the semen of seropositive bulls and venereal transmission postulated but not demonstrated[3].

In humans Q fever causes an acute pneumonia and hepatitis and a chronic endocarditis. In animals infections are often inapparent but cattle and small ruminants may suffer abortion, endometritis and infertility. Repeated occurrences of symptoms may occur[2].

Q fever has a world wide distribution but is not found in New Zealand. It has not been reported in Singapore[4].

Diagnosis can be based on serological tests (complement fixation, ELISA, indirect fluorescence) or isolation of the organism[2].

Release assessment: In view of the possible transmission of Q fever in bull semen, a check of the status of the ram by serological testing is appropriate.

1. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary Medicine, 9th Edition, WB Saunders, London, 2000. pp1275-6.
2. OIE. Manual of Standards for Diagnostic Tests and Vaccines, 4th Edition, 2000. Chapter X.3.
3. Kruszewska D,Tylewska-Wierzbanowska S. Res vet Sci 62, 299-300,1997.
4. OIE. HANDISTATUS II Annual Animal Health Status, 2000

6.2.8 Brucellosis (Brucella melitensis)

Brucella melitensis (3 biovars) is the principal cause of brucellosis is sheep and goats.

The disease in animals is characterized by abortion, retained placenta, orchitis, epididymitis and sometimes, arthritis. It is a serious zoonosis[1,2].

Spread of the disease is through the reproductive tract secretions and milk of affected and non-symptomatic female carrier animals. In spite of the affect on male organs, the male is not considered to be involved in the spread of the disease[1].

The disease is widely distributed in the world but northern Europe, South East Asia, Australia and New Zealand are believed to be free[2]. It is not reported in Singapore[3].

Release assessment: In view of the known distribution of the disease and Singapore's claim of freedom, the ram is not considered to present a risk.

1. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary Medicine, 9th Edition, WB Saunders, London, 2000.
2. OIE. Manual of Standards for Diagnostic Tests and Vaccines, 4th Edition, 2000. Chapter 2.4.2.
3. OIE. HANDISTATUS II Annual Animal Health Status, 2000.

6.2.9 Other diseases

Caseous lymphadenitis caused by *Corynebacterium pseudotuberculosis* , ovine epididymitis caused by *Brucella ovis* and paratuberculosis caused by *Mycobacterium paratuberculosis* were not considered any further because they are endemic in New Zealand[1] and therefore not constitute grounds for denying entry of the imported material.

1. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary Medicine, 9th Edition, WB Saunders, London, 2000. pp 727, 882, 920.

7. Risk Estimation

In this section the risks of the introduction of the disease hazards discussed above are summarized and where appropriate, risk management measures to reduce the risk to negligible levels are proposed. As indicated in the discussion on methodology above, the primary focus is on the state of health of the ram and secondarily on the potential for semen to act as a vehicle for the release of disease into New Zealand. The probability component of the risk equation is the primary consideration on the grounds that costs of keeping unwanted organisms out of New Zealand is likely to much less than having to deal with them after introduction whether by official action to control and eradicate them or by additional costs that they may pose on farming.

Some aspects of the ram's origins and history are important in making judgements about probabilities. Its length of residence in Singapore is important in respect of those diseases that are characterized by incubation periods of less than 18 months and acute or subacute clinical courses. Singapore has an established and respected government veterinary service and a small population of cattle and goats that might be reservoirs for disease. The Singapore Zoological Gardens have a good reputation for the curation of their holdings and health supervision. However, many of the claims of status in respect of specific diseases are based on absence of observation of clinical disease rather than active surveillance.

In contrast, the health history of the ram in China is based to a large extent on hearsay and can not be verified. Thus any estimate of probability has an element of uncertainty that dictates a conservative approach.

It is noteworthy that the ram is 6 years old and was in good health at the time of collection of the samples. He was of an age at which most but not necessarily all animals affected with the disease hazards of concern will have expressed themselves. He is still alive and in good health at the present time.

7.1 The risk of the introduction of the OIE List A diseases

As discussed above, the incubation and infectivity periods of the OIE List A diseases are less than the period of residence of the ram in Singapore and therefore the status of

Singapore is of prime relevance in estimating the probability of infection. Singapore is recognized by OIE as free of FMD and rinderpest. The nature and known geographic distribution of vesicular stomatitis, peste des petits ruminants, Rift Valley fever and sheep and goat pox make it probable that Singapore's claimed freedom is accurate and verifiable. The risk of the introduction of any of these diseases through the use of any of the tissues of the ram is negligible.

While the same can be said for the classical bluetongue, the clinical manifestation of disease can be variable. Because bluetongue exists in the South East Asian region and the existence of competent *Culicoides* vectors in Singapore is unknown, it is recommended that all of the sera from the ram be tested serologically for bluetongue antibodies. If titres are found, their level and whether they are rising, falling or remaining stable will be significant in interpreting their significance. Since the maximum period of infectivity is 60 days (OIE international Animal Health Code), only titres indicative of a recent infection may be significant.

7.2 Risk of the introduction of diseases associated with reproduction

The diseases considered in this section are contagious agalactia, enzootic abortion, *Salmonella abortus-ovis*, Q fever and *Brucella melitensis*. With the exception of Q fever, Singapore's claimed freedom, the nature of the clinical diseases, he known distribution in the world, the absence of a significant susceptible population in Singapore, the fact that the ram has not been in a flock of his own kind for at least 18 months and has not been sexually active all lead to the conclusion that the risk of introduction of these disease is negligible.

While the same can probably be said for Q fever, the fact that latent infections can occur and the agent has been demonstrated in cattle semen suggests that a check on the status of the ram by serological testing would give additional confidence of a negligible risk.

7.3 Risk of the introduction of the diseases characterized by long incubation periods

Scrapie, maedi-visna and pulmonary adenomatosis fall into this category. The issue with respect to this ram is the unreliability of its history of exposure to disease prior to its arrival in Singapore. While the probability of infection is likely to be low in view of the ram's age and freedom from symptoms, any estimate of probability has a

significant level of uncertainty. For this reason a cautious approach is proposed and it is recommended that risk management measures similar to those used for previous importations of sheep into New Zealand be used to reduce the risk to negligible levels.

Satisfactory tests for maedi-visna exist but there are no laboratory based tests for scrapie and pulmonary adenomatosis. The risk reduction measures should include:

- Serological testing of the ram's sera for maedi-visna (agar gel diffusion, ELISA) and *Coxiella burnetti* (indirect immunofluorescence, ELISA, complement fixation).
- Inoculation of sentinel goats intra-cerebrally with homogenized mesenteric lymph node from the ram and observation in containment for a period of three years
- Ewes inseminated with the ram's semen and their offspring held in containment for three years.
- Post-mortem examination of any sheep dying during the quarantine containment period.
- Post-mortem examination of any sentinel goats that die during the quarantine period or are destroyed at the end of it.

The protocol is outlined on MAF's website (http://www.maf.govt.nz/biosecurity/pests-diseases/animals/risk/risk-analysis/risk0016.htm).

7.4 The risk of the introduction of disease through the inoculation of goats with tissue from the ram

One of the measures to test for possible infection of the ram with scrapie is to inoculate goats intra-cerebrally with a homogenate of the mesenteric lymph node tissue. This section is an assessment of the risk of release of an unwanted organism other than the scrapie agent by this method.

One disease hazard additional to those listed above was identified, namely border disease or hairy-shaker disease caused by a pestivirus (family Flaviviridae) closely related to the bovine virus diarrhoea and classical swine fever viruses[1]. Seronegative persistently infected animals are the principal source of infection in a flock. Because the disease is endemic in New Zealand, it was not considered further.

Subject to the testing and containment measures recommended in the discussion in sections 7.1, 7.2 and 7.3 above, the risk of transmission of infection by this means is negligible.

1. OIE. Manual of Standards for Diagnostic Tests and Vaccines, 4th Edition, 2000. Chapter X.10.

Documents attached

I. Ministry of Agriculture and Forestry: Permit to Import Animal Products,17.11.2000.
II. Ministry of Agriculture and Forestry: Biosecurity Authority and Clearance Certificate, 25.1.2001.
III. Ministry of Agriculture, Peoples Republic of China: Animal Quarantine and Export Certificate, 11.9.1998.
IV. CSIRO Animal Health, Australian Animal Health Laboratory: Results of bluetongue virus antibodies tests, 16.12.1998.
V. Agri-food and Veterinary Authority of Singapore: Veterinary Certificate, 18.1.2001.
.

Ministry of Agriculture and Forestry, New Zealand
Te Manatu Ahuwhenua, Ngaherehere, Aotearoa

PERMIT TO IMPORT ANIMAL PRODUCTS

This permit is issued under the Biosecurity Act, 1993. Any queries, please contact Import Management, MAF Biosecurity Authority, P O Box 2526, Wellington, (Phone 64 4 4983624, Facsimile 64 4 4744132)

Authorising Officer : Kerry Mukuwera on 17 Nov 2000

for Chief Technical Officer, Ministry of Agriculture and Forestry, New Zealand, acting under delegated authority.

Permit for : Diverse Agricultural Holdings
70 Te Hau Road
RD 8, Matahiwi
Masterton
New Zealand
Attention : Andrew Frontin-Rollett

Permit No : 2000010925
Replaces Permit No : N/A
Expiry Date : 01 May 2001
No of Consignments : Single
Import Purpose :

Establishment No :

Shipped From : Singapore

Exporter Name : Singapore Zoological Gardens
80 Mandai Lake Road
Singapore

Country of Origin : Singapore

Product To be Exported : Biologicals - Restricted

Description of Items	Quantity	Measure
Argali sheep: semen, mesenteric lymph nodes, ear tissue	Unlimited	Units

Import Health Standard

SHETISIC.SIN, 13 Nov 2000, SHE - Argali sheep tissues, blood & semen into a transitional facility

Ministry of Agriculture and Forestry
Te Manatu Ahuwhenua, Ngaherehere

B:2001/4661

MAF Quarantine Service

Biosecurity Authority/Clearance Certificate

Pursuant to Sections 25 and 26 of the Biosecurity Act 1993

CUSMOD Release Number: AF100100085733 **Consignment Reference Number:** C2001/3214

Each Authority contained in this document identifies the goods that are covered by the Authority, a Transitional Facility that you are authorised to take the goods to, and any conditions which the authorisation is subject to.

Removal of these goods to a place other than the Transitional Facility authorised, or otherwise than in accordance with the conditions specified, is an offence.

Any Clearance or Authority that is contained in this document, and that relates to forest produce, also constitutes permission to remove the forest produce under the Forest Produce Import and Export Regulations 1989.

All Biosecurity Requirements Met?

NO

Authority issued to: Owens International Freight
590 Great South Road, Penrose , Auckland

Importer: Diverse Agricultural Holdings
Masterton

Agent: Owens International Freight
Auckland

Arrival Method: **Flight:** SQ285 **Date:** 25/01/2001

IDENTIFIERS:

Bill of Lading: 61899016842 AWB# NG433272.

AUTHORITY:

To be taken to: AgResearch Centre - Ruakura, East Street, , Hamilton

For: Post entry quarantine under MAF Quarantine supervision

By: Biosecurity Officer, North , Hamilton

Authority Conditions:

To be taken to AgResearch Reproductive gourp - Ruakura

Authorising Inspector: Harris, Pauline **Location:** Auckland - Airport **Date:** 25/01/2001

GOODS COVERED BY THIS AUTHORITY:

Type:	Country:	Description:	
1 Biologicals	SINGAPORE	samples (animal material), various, 12.000 piece(s)	**Bill of Lading:** 61899016842
2 Animals	SINGAPORE	Ovine, Semen, 45.000 straws	**Bill of Lading:** 61899016842

Issued by: Harris, Pauline
Date: 25/01/2001

Location: Auckland - Airport **Signed:**

Page 1 of 1

19/03/2001 11:19 +64-4-3838358 DIVERSE AGRIC HLDGS PAGE 05

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副本
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中华人民共和国农业部

动物检疫证书

ANIMAL QUARANTINE CERTIFICATE

MINISTRY OF AGRICULTURE OF P.R CHINA

CAPQ

编号 No. ____

发货人及地址
Name & Address of Consignor BEIJING ZOO

受货人及地址
Name & Address of Consignee ____

动物种类
Kind of Animal ____

品种
Species ____

特征
Characteristics ____

数量
Quantity Declared ____

产地
Place of Origin ____

发货口岸
Port of Despatch ____

到达口岸
Port of Destination ____

发货/到货日期
Date of Despatch/Arrival ____

运输工具
Means of Conveyance ____

合同/合约号
No. of Contract ____

检疫日期
Date of Inspection ____

签证日期 Date of Issue ____

印章
Stamp

兽医官 Veterinary Officer ____

签名 Signature ____

[illegible]不承担签发本证书的任何财经责任。

Export Certificate

I, Ma Guiping, a Veterinary Officer employed by the Government of the Peoples Republic of China certify after due enquiry that I am satisfied that the seven (7) animals listed below:

1. Were derived from premises which have been free from Foot and Mouth disease, Rinderpest, Contagious Bovine Pleuropneumonia, Peste des petits ruminants, Contagious Caprine Pleuropneumonia, and Bluetongue for at least 12 months prior to export.

2. Have been kept in captivity in the said premises for at least 6 months prior to export or since birth and the animals have been kept in isolation in a quarantine station for at least 40 days prior to export

3. Are from an area in which infectious and contagious animal diseases are under control and where no case of Trypanosomiasis, Anaplasmosis, Johne's Disease, Rabies, or Brucellosis has occurred in the last 3 months prior to export.

4 Have not been vaccinated with any vaccine within 30 days of export.

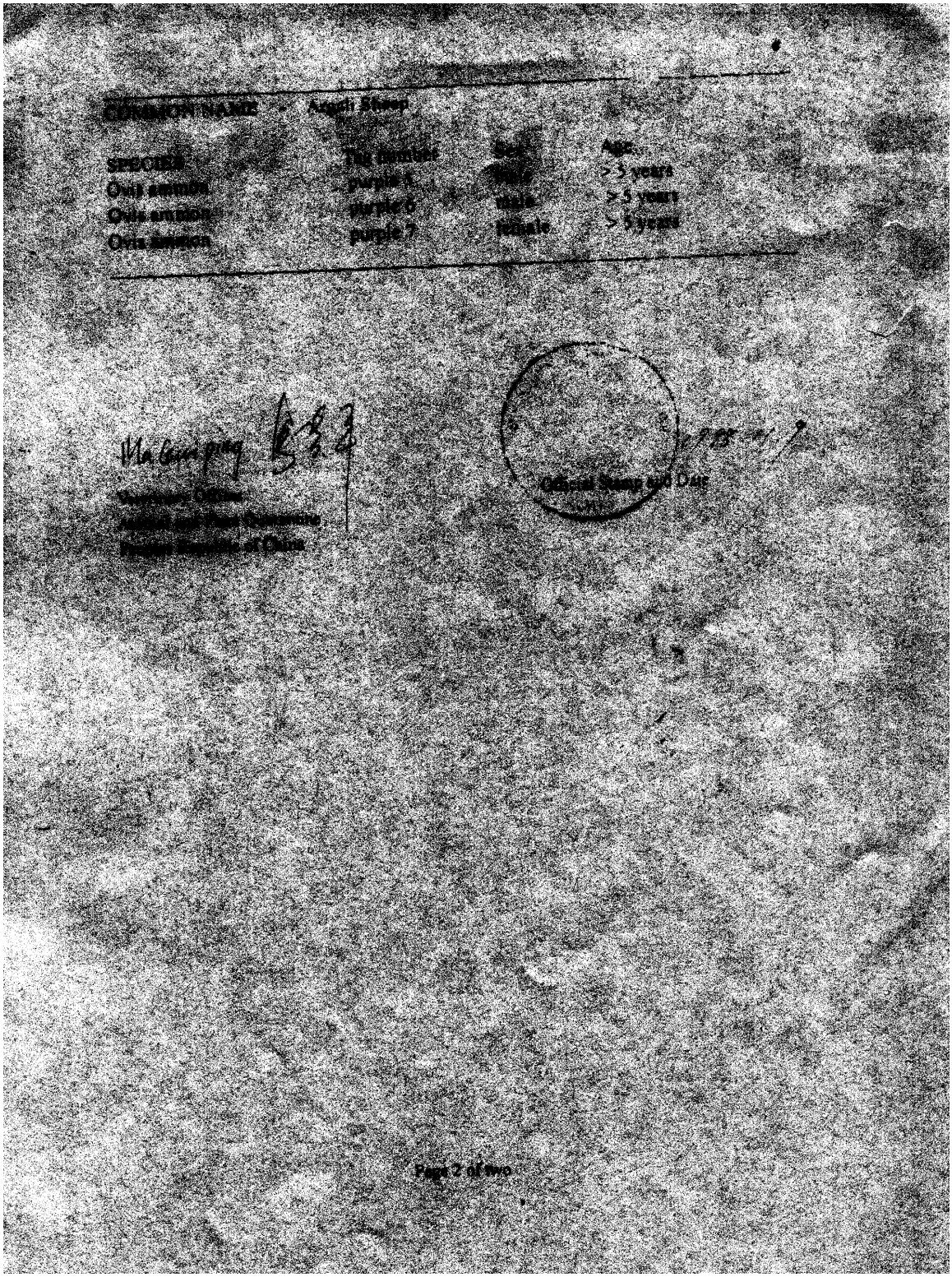
5. Have been examined and found to be healthy and free from any clinical signs of infectious or contagious disease (including ectoparasites) at the time of export.

6. Were protected from insect vectors during the pre-export quarantine period and transportation to the place of shipment

---

COMMON NAME - Bharal or Blue Sheep

SPECIES	Tag number	Sex	Age
Pseudois nayaur	purple 1	male	> 5 years
Pseudois nayaur	purple 2	male	> 5 years
Pseudois nayaur	purple 3	female	> 5years
Pseudois nayaur	purple 4	female	> 5 years


COMMON NAME : Argali Sheep
SPECIES
Ovis ammon
Ovis ammon
Ovis ammon
The number
purple 1
purple 6
purple 7
Sex
male
female
Age
> 5 years
> 5 years
> 5 years
Official Stamp and Date
Page 2 of two

04-APR-2001 14:04 FROM TO

IV

CSIRO Animal Health
Australian Animal Health Laboratory (AAHL)

5 Portarlington Road, Geelong Victoria, Australia
Private Bag 24 Geelong Vic 3220
Ph 03 5227 5000 Int +61 3 5227 5000
Fax 03 5227 5555 Int +61 3 5227 5555
www.ah.csiro.au email ...@ah.csiro.au
Chief: Mike Rickard

Diagnostic Specimen Testing

Copy for your information of final report

SAN: 98-1761
Date: 16 December 1998

Attn Dr Sasha Herbert
Singapore Zoological Gardens
Mandai Lake Road

SINGAPORE
65 2672974

Sender Dr Sasha Herbert
Singapore Zoological Gardens

Ref:

Owner
Singapore Zoological Gardens

Examination Blue Tongue Virus antibodies - EN #5 Microchip #00-01BD-4FBB, EN #6 Microchip #00-01BD-5479, EN #7 Microchip #00-01BD-6938, EN #1 Microchip #00-01BD-4ABE, EN #2 Microchip #00-01BD-543E, EN#3Microchip 00-01BD-595F, EN#4 Microchip 00-01BD-5C5E,

Sample Identification:
EN #5 Microchip #00-01BD-4FBB, - Argali, male
EN #6 Microchip #00-01BD-5479,
EN #7 Microchip #00-01BD-6938,
EN #1 Microchip #00-01BD-4ABE,
EN #2 Microchip #00-01BD-543E,
EN#3Microchip 00-01BD-595F,
EN#4 Microchip 00-01BD-5C5E,
Sample collection date. Not stated
Received at AAHL. 8 Dec 98

Bluetongue serology
The sera were tested for antibody to bluetongue virus by CELISA. Serum dilution was 1 in 10
Results: All seven sera were negative for antibody to bluetongue virus.

Yours faithfully

Deborah Middleton
for Head of Laboratory

Page 1 of 1

AVA

Agri-food & Veterinary Authority of Singapore
City Veterinary Centre
25 Peck Seah Street, 2nd Storey, Singapore 079315
Tel: (65) 2270670 Fax: (65) 2276403

VETERINARY CERTIFICATE

I, **EUGENE CHAN, DVM**, being a Singapore Government Veterinary Officer certify that:

1. **COUNTRY FREEDOM**

   1.1 Singapore is free of foot and mouth disease; vesicular stomatitis; rinderpest; peste des ruminants, sheep and goat pox, bluetongue and Rift Valley fever.

2. **RESIDENCY AND AGE**

   After due enquiry I am satisfied that:

   2.1 the donor for this consignment has resided for the last 12 months at Singapore Zoo,

   2.2 at the time of collections the donor was aged 6 years.

3. **FLOCK OF ORIGIN**

   3.1 For the 6 months preceding the collection of tissues and semen, none of the ruminants at Singapore Zoo have been placed under any official quarantine restrictions.

4. **IDENTIFICATION OF DONOR**

   4.1 The donor was identified using a Trovan Transponder No.: 00-01BD-4FBB (ISIS accession # G6154).

5. **PRE-COLLECTION**

   5.1 During the 30 day pre-collection isolation period and throughout the collection period, the donor and all in-contact animals remained isolated from any other animal not of equivalent health status.

   5.2 The tissues, semen and blood in this consignment have been securely stored in sealed containers until the time of export.

   5.3 The final audit and sealing of the transport containers for export was performed under my supervision. The containers were sealed with official seals bearing the mark or number appearing in section I of this certificate.

Head Office
5 Maxwell Road #02-03-00 Tower Block MND Complex Singapore 069110
Fax: (65) 2206068

6. **COLLECTION**

6.1 The following samples were collected from the donor :

6.1.1 At the time of tissue collection, or during the 60-day post-collection period the donor has undergone surgery to remove 4 grams of mesenteric lymph node i.e. at least two large mesenteric lymph nodes. These nodes were placed in a sterile container, labelled, the air excluded and stored at a minimum temperature of –70°C.

6.1.2 Only sterilised* flasks and fresh nitrogen not previously used for any other purpose was used for storage of the tissues.

[*Sterilised means autoclaved, processed by dry heat or subject to 5% formalin treatment in accordance with recognised international standards.]

6.1.3 Blood samples were collected 30 days before tissue collection and 30 days after. The sera was stored frozen – (-20 °C) or stored in the same conditions as 6.1.2.

6.1.4 The lymph nodes, tissues, blood and semen have been identified to the donor.

6.1.5 The containers were stored under secure conditions until the final audit and their sealing for export to New Zealand. The containers were sealed with official seals bearing the marks or numbers appearing in section I of this certificate.

7. **POST-COLLECTION HEALTH STATUS**

During the collection period, and for the 60 days following collection, the donor, and all in-contact animals have remained healthy.

8. **TRANSPORT TO NEW ZEALAND**

8.1 Immediately prior to despatch, the containers containing the tissues, semen and blood were sealed under my supervision and the numbers of the seals recorded on section I the zoo-sanitary certificate.

8.2 The tissues, semen and blood for export to New Zealand were sent to the port of embarkation under quarantine conditions.

18 JAN 2001

.....
Government Veterinary Officer

.....
Date and Official Stamp

Name and address: Eugene Chan, DVM

[illegible] VETERINARY CENTRE
[illegible] STREET
[illegible] 3315
[illegible] FAX: 2276305