Discussion paper

IMPORTATION OF LABORATORY RATS AND MICE AND REPRODUCTIVE MATERIAL

Background

Conditions for importation of laboratory rats and mice were reviewed in 1998 and the current conditions were issued in June 1999 (AQPM 1999/043). Since that time, several requests have been made for importation of mouse embryos and semen with a view to gaining access to special strains of mice for research purposes.

The importation of embryos, ova and semen in lieu of live animals is promoted as being inherently safer from a biosecurity perspective. Many agents of concern are not transmitted vertically via *in-utero* transmission to the embryo or foetus. Many may not be transmitted via semen. Thus, there may be a lower risk than live animal importation. This document reviews the disease risks involved in importation of mouse embryos, ova and semen and discusses options for extending the current conditions for live laboratory rats and mice to include these products. This review is limited to the species *Mus musculus*, *Rattus rattus* and *Rattus norvegicus*. Its scope is also limited to animals kept under laboratory conditions - rats or mice reared as pets could carry a range of pathogens significantly wider than animals reared in laboratories.

This document also reviews the use of source colony disease status (as an alternative to post arrival quarantine and testing) as a means of ensuring that imported animals, embryos, ova or semen are free of the agents of concern. In the context of importation of live laboratory rats and mice, it discusses this alternative option.

Hazard identification

Hazards identified for consideration in respect of live laboratory rats and mice were as follows:

- Hantaviruses
- Lymphocytic choriomeningitis virus
- Ectromelia virus
- Rabies virus

This review has not found any additional agents, which should be considered and assessed at this time.

It should be noted that importing laboratories may also be concerned about diseases which are present in Australia but which do not occur in their colonies. Such laboratories are free to make private contractual arrangements for testing or certification which is additional to that required officially.

What is a "colony"?

For the purpose of this review, the term "colony" refers to the entire group of animals that are in contact with each other. The members of the group may be in different cages within the same room

provided they share airspace, handlers and equipment and there is no air filter or any similar physical barrier between animals.

Disease Agents of Concern

1 Hantaviruses

The agent

Hantaviruses are members of the arthropod-borne family Bunyaviridae. Hantaan virus is the name given to a member that caused Korean haemorrhagic fever, a syndrome recognised during the Korean War and characterised by acute fever, shock, haemorrhage and renal failure. Collectively, the World Health Organisation (WHO) has grouped hantaviruses as the viruses of haemorrhage fever with renal syndrome (HFRS).

Rodents are the primary reservoir hosts of Hantaan and related viruses. In the laboratory, the rat is the primary animal associated with the spread of hantaviruses although isolation from *Mus musculus* has been occasionally reported (Harkness and Wagner 1995).

Although not likely to cause disease in rodents, hantaviruses may cause serious disease and death in humans. Laboratory personnel have contracted disease from infected laboratory rats in Japan, Belgium, France, Korea, and Singapore (Harkness and Wagner 1995).

Likelihood of entry into Australia

Biosecurity Australia is not aware of any reports of hantaviruses being transmitted via embryos or semen.

A literature search has not revealed any reports of positive finding in Australia of hantaviruses in rodents or humans. The likelihood of entry of hantaviruses with mice from certified laboratory animal sources is extremely low given quarantine considerations in such facilities and that mice (*Mus musculus*) have only very occasionally been associated with hantavirus infection.

The risk would appear to be higher in the reproductive material of rats than of mice since hantaviruses have only very occasionally been found in the mouse. In light of the current international trade in these two species it would seem the importation of mouse embryos will, when permitted into Australia, occur at a much greater rate than rat embryos. The likelihood of entry of hantaviruses during unrestricted importation of live rats or rat genetic material from certified laboratory facilities is regarded as very low.

Current requirements for importation and release from quarantine of live laboratory rats and mice include post-arrival testing for hantaan virus using a sample size sufficient to detect a 5% prevalence of infection at a 99% confidence level. In laboratory colonies, it is predicted that the actual seroprevalence of hantavirus antibodies within an infected colony would likely be much higher within a few weeks of the agent being present. Provided the colony is on a regular testing programme for hantaviruses, it is proposed that a sample size sufficient to detect a 25% prevalence of infection (at a 99% confidence level) is appropriate.

Proposed risk management option for laboratory rats and mice or reproductive material

A Pre-export testing: The proposed requirement is that:

1. The premises of origin have been free of any evidence of hantavirus infection for the past 12 months; and

2. The source mouse or rat colony has been regularly tested at intervals not exceeding 6 months for hantavirus infection; and
3. The source mouse or rat colony has been sampled and tested within the last 6 months in a manner which gives a 99% confidence that less than 25% of the animals have demonstrable antibodies to hantaviruses (dates and test information to be provided); and
4. No new animals have been introduced to the colony later than 4 weeks prior to the last sampling up to the time of export or collection of genetic material for export.

B Post arrival testing: Where for any reason the source colony cannot be tested, the following alternative requirements for testing after arrival may be used:

1. The imported rats/mice/genetic material must be consigned directly into an AQIS approved quarantine premise and remain there until tested as follows.
2. a) The imported rats and mice or derived animals and their progeny will be eligible for release from quarantine only if they (the imported animals and progeny) have been sampled and tested in a manner which gives a 99% confidence that less than 5% of the animals have demonstrable antibodies to hantaviruses (dates and test information to be provided); or

   b) The imported rats and mice or derived animals and their progeny have been regularly tested at least 3 times, at intervals not exceeding 6 months, in a manner which gives a 99% confidence that less than 25% of the animals have demonstrable antibodies to hantaviruses and have shown negative results in all such tests (dates and test information to be provided); and
3. Biosecurity of the colony including any introduced animals must be maintained until testing is complete.

References

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2 Lymphocytic choriomeningitis virus (LCMV)

The agent

Lymphocytic choriomeningitis virus belongs to the genus Arenavirus in the family Arenaviridae. The natural host for the LCM virus is the wild mouse, in which the prevalence of infection may approach 100%. The virus produces a clinically inapparent lifelong infection. Natural infections occur in wild mice (*Mus musculus*), guinea pigs, monkeys and man. Infection can be readily transmitted to hamsters.

Lymphocytic choriomeningitis may be transmitted from infected animals to humans through direct or indirect contact with faeces, urine, infected murine tissue, or by biting (Harkness and Wagner

1995). LCM in man causes an aseptic meningitis, encephalitis, or meningoencephalitis, with a mortality of less than 1%.

In mice, on the basis of clinical signs, viscerotropic and neurotropic strains exist. Syndromes subsequent to infection depend on age, route of infection, strain of virus and strain of mouse (Lehmann-Grube 1982).

In utero transmission is common in infected mouse populations. The virus is passed in the urine, saliva, milk, and faeces and enters susceptible individuals via traumatized skin, the conjunctiva, or respiratory passages. Blood sucking arthropod vectors, such as ticks, lice and mosquitoes, as well as dust may be means of transmission (Harkness and Wagner 1995).

In colonies of mice carrying LCM virus, individuals are infected before birth, either as eggs in the ovary or early embryos (Mims 1966). In a subsequent study (Mims 1969) found that when 7-8 day pregnant WEHI mice were injected with relatively large doses of LCM virus intravenously, foetuses were infected by the second day, probably by spread from the foci in primary giant cells across Reichert's membrane to the yolk sac and amnion. Infection spread by contiguity into the foetal organs and there was foetal death and resorption by the fourth day.

Typically, vertical transmission following *in utero* or neonatal infection of mice results in persistent tolerant infection. In such cases there will be a negative serological response.

The virus is widespread in Europe and the Americas. In Australia, natural LCMV infection has been reported in north-eastern NSW (Smith *et al* 1993). LCMV antibodies were detected at 3 sites at Narrabri and Moree, out of 14 sites examined in Qld, NSW, Vic and SA. In a survey in 1989, 17/33 samples from these 3 sites were positive; in 1991, 14/47 were positive. The other 11 sites were negative when sampled (generally once only) during the period 1987-1991. Populations at the 3 positive sites were sampled again in late 1994 and 46/102 mice were sero-positive (Smith 2002, pers comm). In this instance LCMV was isolated from the mice. The Australian LCMV isolates were all shown to be antigenically identical to prototype LCMV. They were shown to be of differing virulence for laboratory mice of different strains. They have not yet been fully characterised.

The likelihood of entry of LCM virus through the importation of mouse reproductive material during unrestricted importation of live mice or mouse genetic material from certified laboratory facilities is regarded as very low. Generally such facilities undertake routine testing for LCMV using ELISA or IFA testing. In one study, 1000 serological investigations of laboratory animal colonies originating from 10 different European countries revealed no infections with lymphocytic choriomeningitis virus (Kraft and Meyer 1990).

The current requirements for post-arrival testing for LCM virus were presumably instituted at a time when the status of LCM in Australia was not as clear as it is now. Given the fact that LCMV is present in Australia and there are no measures in place to limit its possible spread, it is proposed that it not be the subject of quarantine requirements. (This does not preclude importers applying their own requirements that source colonies be free of the organism.)

References

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3 Ectromelia virus

The agent

Ectromelia virus is a member of the orthopoxvirus genera, of the family Poxviridae. It causes mousepox and affects mice only. Poxviruses are the largest and most complex of all viruses. In terms of cytopathogenicity they have a predilection for epidermal cells.

Ectromelia virus is regarded as a potentially useful agent for the biological control of mouse plagues in Australia. It is mainly for this reason that its inadvertant introduction into Australia is regarded as undesirable. If it were inadvertantly released, the widespread development of immunity or tolerance amongst wild populations of mice could compromise the potential use of the virus under properly controlled conditions as a biological control agent.

It is of note however, there has been only one report of finding this virus in wild mice not associated with colony mice and this was questionable (Fenner 1982).

Epizootics of ectromelia virus infection occurred in the USA in laboratory mice colonies in 1979-80 (Wallace and Buller 1986). Pathogenesis in mice varies with the strain of the virus, route of infection and the genotype of the host. During natural infection (contamination of skin abrasions with the virus), viral replication occurs in the skin followed by a primary viraemia at three days and skin lesions at seven days. A secondary viraemia and rash begins at days five and ten respectively, with extensive viral replication and cell destruction in the liver and spleen. In acute fatal cases there may be no clinical signs of infection. Mice surviving beyond ten days develop a characteristic rash and conjunctivitis followed by necrosis of the appendages and in some cases, amputation of the tail, limbs and ears (Wallace and Buller 1986).

Harkness and Wagner (1995) state that humans are not susceptible to infection by ectromelia virus. Fenner (1982) had previously found that attempting to infect humans with ectromelia had been virtually asymptomatic and HI antibody titre unaffected. Fenner (1982) also cited a situation where "hundreds of thousands" of humans had been inoculated subcutaneously with ectromelia virus either alone or mixed with rickettsiae and no subsequent local or general reaction of any significance was reported.

Mims (1969) injected (in the footpad) 10 seven day pregnant mice with an attenuated Hamstead strain of ectromelia virus. The resulting infection was subclinical in the mothers but there was extensive growth of virus in the placentae and infection of the foetuses that died either in-utero or

soon after birth. Fluorescent antibody staining showed widespread virus throughout the bodies of foetuses.

Likelihood of entry into Australia

In the above cited study of 1000 serological investigations of laboratory animal colonies originating from 10 different European countries (see page 4), only two colonies of mice were infected with ectromelia virus (Kraft and Meyer 1990).

The likelihood of entry of ectromelia virus through the importation of mouse reproductive material could be significant in the light of the above work demonstrating infection of foetuses. However, the likelihood of entry is regarded as very low, providing that importation is done from research facilities that have regularly shown negative results with ELISA testing. The ELISA test for this virus is regarded as being sensitive and is commonly used as the primary serologic test (Harkness and Wagner 1995).

Inapparent infections and low prevalence of enzootic disease may create major problems in establishing a diagnosis of mousepox (Institute for Laboratory Animal Research 1991). In certain outbreaks in the USA in the early 1980s, ectromelia virus was present for long periods before recognition, although this may have preceded the development of the more sensitive ELISA test. Current requirements for importation and release from quarantine of live laboratory mice include post-arrival testing for ectromelia virus using a sample size sufficient to detect a 5% prevalence of infection at a 99% confidence level. It is proposed to use the same sampling level for the testing of colonies for pre-export testing.

Proposed risk management option for laboratory rats and mice or reproductive material

A Pre-export testing: The proposed requirement is that:

1. The premises of origin have been free of any evidence of ectromelia virus infection for the past 12 months; and
2. The source mouse colony has been regularly tested at intervals not exceeding 6 months for ectromelia virus infection; and
3. The source mouse colony has been sampled and tested within the last 6 months in a manner which gives a 99% confidence that less than 5% of the animals have antibodies to ectromelia virus (dates and test information to be provided.)
4. No new animals have been introduced to the colony later than 4 weeks prior to the last sampling up to the time of export or collection of genetic material for export.

B Post arrival testing: Where for any reason the source colony cannot be tested, the following alternative requirements for testing after arrival may be used:

1. The imported mice/genetic material must be consigned directly into an AQIS approved quarantine premise and remain there until tested as follows.
2. The imported mice or derived animals and their progeny will be eligible for release from quarantine only if the colony has been sampled and tested in a manner which gives a 99% confidence that less than 5% of the animals have demonstrable antibodies to ectromelia virus (dates and test information to be provided - this testing should be conducted within 3 months of importation);
3. Biosecurity of the colony including any introduced animals must be maintained until testing is complete.

References

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4 Rabies virus

The agent

Rabies is caused by a virus belonging to the lyssavirus genus of the family Rhabdoviridae. In addition to classical rabies viruses (which are now classified within serotype 1 of the group), this genus contains a number of antigenically related viruses, some of which cause rabies-like disease in vertebrate animals. Australian bat lyssavirus is the only know member of the lyssavirus genus which is endemic in Australia. It is found in both insectivorous bats and flying foxes.

Rabies is present in most of Europe except the United Kingdom, Ireland and parts of Scandinavia. Rabies is also prevalent in Africa, the Middle East, most of Asia, and in North, Central and South America. Japan, Singapore, most of Malaysia, Papua New Guinea, most of Indonesia including Irian Jaya, New Zealand and the Pacific Islands are free of the disease (Geering *et al* 1995).

Rabies is an important zoonosis in many countries and if it became established in Australia, would have very significant consequences (Krebs *et al* 1998). There are a number of different possible scenarios under which an incursion of rabies could affect Australia. On the one hand, there could be a less consequential incursion whereby the disease was eradicated before it became widespread. On the other, establishment in native animals under circumstances that allowed the disease to become endemic would have profound and long-term social and economic consequences.

In the present context of laboratory rats and mice, any incursion of the virus would be likely to be detected and relatively easily eliminated at an early stage and in particular before it had gained access to a suitable species which might allow a wider dissemination of disease.

Likelihood of entry into Australia

The likelihood of entry of rabies virus via live rats or mice or their genetic material is regarded as extremely low. Although the virus grows in embryonic tissue of a range of different species, there are no reports of it being isolated from embryos of infected animals of any species, nor of its transmission through artificial insemination or embryo transfer. Transmission of rabies to the foetus *in utero* has been reported in skunks, bats, cows and man (Greene 1998) but this is not well documented. The likelihood of transmission of rabies virus via rat or mouse embryos or semen is regarded as very low.

Proposed risk management option for laboratory rats and mice or reproductive material

The proposed risk management measure in respect of rabies is that the source colony be certified as having been free of any evidence of rabies for the 6 months prior to export of live animals or collection of genetic material.

References

Geering WA, Forman AJ and Nunn MJ (1995). *Exotic Disease of Animals - a field guide for Australian veterinarians.* Australian Government Publishing Services, Canberra.

Krebs JW, Long-Marin SC and Childs JE (1998). Causes, costs and estimates of rabies postexposure prophylaxis treatments in the United States. *J Public Health Mgt Pract* 4: 57-63.

Greene, CE (1998). *Infectious Diseases of the Dog and Cat* 2nd Edition. WB Saunders.

Draft conditions

[See attached.]