

Importation, quarantine and monitoring of laboratory animals, particularly rodents, for issue in Australia

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Introduction

The availability of laboratory animals which are disease and pathogen free and which do not have antibodies which are indicative of past exposure to agents of concern is critical to laboratory services. The course of acquisition from production facilities of known status, shipping under appropriate security and maintenance in housing of appropriate isolation presents its own demands on animals and users.

This paper will not deal with the logistics of handling, housing and airfreight of laboratory animals. Details of requirements are available from the International Air Transport Association (IATA) or from freight forwarding agents.

The number of specific genetic and standardised lines identified for particular tasks means that they are traded around the world in significant numbers. Disease control and welfare issues require particular housing arrangements, attention, and continued monitoring.

The nature of the laboratory animal, methods of housing and care and its relationship with humans create complexity in establishing disease freedom. This usually involves sample testing.

At the national and international levels there are obligations regarding animal health and welfare which affect this matter. Australia's traditional stringent quarantine arrangements which have successfully excluded many livestock diseases are also employed for laboratory animals.

Quarantine

Quarantine in Australia is the responsibility of the Australian Quarantine and Inspection Service (AQIS), an agency of the Australian Department of Agriculture, Fisheries and Forestry.

Quarantine requirements reflect the known disease status and value of such animals, methods of handling and the facilities in which they are held. They are designed to exclude exotic and zoonotic diseases and those troublesome in laboratory colonies. Current requirements centre on hantavirus, lymphocytic choriomeningitis virus, ectromelia virus and rabies but are mindful of other diseases.

Quarantine policy and legislation

Australia's policy has always been conservative but all quarantine restrictions have been scientifically based. Where there has been inadequate scientific information on which to base decisions the precautionary principle has been applied.

The Quarantine Act 1908, one of the early Acts of the new Commonwealth provides the legislative basis and authorises the making of Regulations (which specify how) and Proclamations (which specify what) regarding quarantine activities and commodities that may be imported. This subordinate legislation is made by the Executive Council (Governor-General and Ministers) but is scrutinised by Parliament. The legislation provides certain delegations and authorities to the Director of Quarantine who can authorise "protocols" or "conditions" which provide flexibility and detail necessary to address biological variation.

Australia has obligations arising from its membership of the World Trade Organisation. The Agreement on the Application of Sanitary and Phytosanitary Measures of the WTO mandates the use of risk analysis and, inter alia, consultation and transparency in quarantine decision making because of the potential for unjustified impediments to trade. The Agreement also mandates the International

Animal Health Code of the Office International des Epizooties (OIE) - the world organisation for animal health as the standard for quarantine.

The Code provides the general basis for international movement of animals, their genetic material and products derived from them while minimising the spread of disease. The Code also provides the basis of inspection, quarantine and certification.

Australian quarantine policy and procedure were recently reviewed by a Quarantine Review Committee chaired by Professor Malcolm Nairn. The Committee recommended an Import Risk Analysis (IRA) process of routine risk analysis (in house) for common routine cases or where there were precedents and of non-routine risk analysis where there were not. The latter involves the formation of Risk Analysis Panels (RAP) of Experts. The mandated process involves consultation with stakeholders at various parts of the process, including the process itself, composition of the RAP and the draft IRA paper, so that it is truly transparent. The AQIS Bulletin advises interested parties of its intention to undertake an IRA, of the progress made and of the availability of the draft IRA.

Methods of risk analysis are addressed in the OIE Code. Much has been written on risk analysis, essentially a step by step evaluation of the risks of each identified disease from the colony of origin to release in the importing country, is done. This may be quantitative, semi-quantitative or qualitative. The Code addresses:

- country factors e.g., the disease status of the export country/facility;
- commodity factors i.e., the capacity of the animal or product to carry the disease(s);
- number of import units (to quantify the risk); and
- risk of domestic exposure (in the country/facility of import).

Risk management is the process by which the risks are addressed e.g. by testing or quarantine.

Quarantine requirements

AQIS has developed guidelines for the approval of countries to export animals and their products to Australia. Current conditions for the importation of laboratory rodents were introduced in December, 1998.

The AQIS permit does not absolve the importer from the necessity of obtaining permission to import under the Wildlife Protection Act 1983, should this be appropriate.

The conditions require the donor colony to be free from the following diseases or infectious agents during the 12 months prior to export:

- hantavirus;
- lymphocytic choriomeningitis virus;
- sendai virus;
- ectromelia virus; and
- rabies.

Importers may wish to test for other disease agents to protect their colonies.

The colony containing the animals for export must be housed in accommodation which precludes access by wildlife, including rodents, and be insect vector proof and free of ticks.

The animals to be exported and the donor colony must have remained clinically healthy and free from infectious and contagious diseases in the 30 days prior to export.

Each animal for export must be examined by an official veterinary officer during the 48 hours prior to loading and be fit to travel and free from evidence of infectious and contagious disease and external parasites. Specific Pathogen Free (SPF) animals are exempt from examination, but certification by an official veterinary officer and the veterinarian in charge of the donor colony of their SPF status must be provided.

Transport should be in a container as specified under IATA Live Animal Regulations. On arrival, all litter in the containers must be destroyed.

Imported animals

The imported rats and mice must be maintained in quarantine premises approved by the Chief Quarantine Officer (Animals). They must be kept in secure containers in a locked building with keys to be held by nominated responsible persons. No transfer of imported rats and mice or offspring is to be made without the permission of the Chief Quarantine Officer (Animals).

A register of all imported rats and mice must be kept by a nominated responsible person. This register shall contain the following information:

- source of animals;
- identity of animals;
- numbers of animals imported, used, born, weaned, transferred, died; and
- cause of death.

All animals on the premises must be immediately identifiable as to their source and quarantine status. Microchips may be used. Advances are being made in national and international standards for microchips so that readers of all brands will be able to read all microchips and registers of microchips will be linked for ready access.

Imported animals may be isolated in microisolators. Non-imported animals may be placed in the quarantine room for use for breeding or as sentinels for immunocompromised imported animals. Animals in contact with imported animals will remain in quarantine until the imported animals are released from quarantine. Husbandry and handling practices, including traffic flows, must be of a standard which ensures the integrity of the quarantine status of the imported animals and thus reduces the likelihood of spread of disease.

If any of the imported or contact animals suffer from or are suspected of illness, or death is suspected to have been caused by hantavirus, lymphocytic choriomeningitis virus or ectromelia virus, AQIS must be notified immediately.

Imported animals must remain in approved quarantine premises during the entire period of their use in research. The imported animals must be kept physically isolated from all other animals in the facility not of the same quarantine status. Any animals (including sentinels) in the quarantine facility that come in contact with imported animals will assume the same quarantine status as the imported animals. Imported animals, must, at the end of their use be disposed of in a manner approved of by AQIS (e.g., autoclaving or incineration).

Release from quarantine

Progeny of imported rats and mice may be released from quarantine to institutions registered by their State or Territory (an institution holding animal ethics clearance) to hold rodents if the above conditions are met.

Except for *Mus musculus*, *Rattus rattus* and *Rattus norvegicus*, permission must be obtained from Environment Australia to transfer rats and mice and their offspring from quarantine premises to other premises.

Progeny of imported animals are eligible for release only if prescribed tests are performed on a statistically valid sample of all the rats and mice at least eight weeks of age and show freedom from the following disease agents:

- hantaan virus - enzyme linked immunosorbent assay (ELISA)
- lymphocytic choriomeningitis virus- ELISA
- ectromelia virus (mice only) - ELISA

Other test methods may be used with prior approval from AQIS.

Several options for sampling of the imported animals are provided:

- one off sampling of imported animals and their progeny in the quarantine room. Sample size must be sufficient to detect a 5% prevalence of infection at a 99% confidence level and no introductions within 30 days of blood collection;
- sampling the colony of imported animals and progeny in the quarantine room on a quarterly basis over the previous 12 months. Each sampling to detect a 30% prevalence at 99% confidence and no introductions within 120 days of release i.e., 30 days before third sampling;
- progeny maintained as a separate biological unit from the imported animals, each sampling to detect a 30% prevalence at 99% confidence and no introductions within 120 days of release i.e., 30 days before third sampling.

For the second and third options, provided the colony is on a quarterly testing program, progeny can be released after two negative quarterly tests at least 30 days after the last introduction.

If any of the animals test positive, the officer in charge of the colony must notify AQIS. No release will be allowed and AQIS will give instructions as to further investigations required or the disposal of the positive animals and those in contact.

Where immunocompromised mice are imported, sentinels may be used, under the following circumstances:

- sentinels (8 to 12 weeks of age, the same species as those in quarantine) must be placed in contact (in the same boxes) with the imported animals on arrival in quarantine for a minimum of 45 days but not more than 120 days prior to testing for the diseases listed above;
- the number of sentinels to be placed in contact with the colony is calculated from the number of animals in the colony prior to adding the sentinels to give 99% confidence of detecting disease if it is present at 5% prevalence. A few additional animals should be added to the colony.

A report containing the test methods, the name of the testing laboratory, and numbers of animals tested must be provided to AQIS before approval for release will be given. The purchasers of animals may require pre-transfer testing for other disease agents.

Premises

AQIS requires imported biologicals and laboratory animals to be kept in approved laboratories. There are requirements regarding location, equipment, waste control and record keeping which are controlled through quality assurance arrangements which may be based on HACCP principles. This method allows the management to develop quarantine security arrangements which best suit their facilities and operations. It might be said that the process is outcome orientated so that the individual method is less important than the result. This suits the wide nature and purpose of laboratories very well and lightens intervention into regulatory measures while allowing the disease control and welfare needs to be met. Where necessary premises and records are audited.

Disease control

Disease control tends to be based on quarantine and the procurement of healthy stock from accredited or reputable sources. Transport in isolators and quarantine or isolation on arrival may be required. Design of housing or vivaria is important in prevention of cross infection. Bacterial diseases may be treated by chemotherapeutics in feed or water. Mycotic diseases are controlled principally by careful purchase and by control of the animal house environment.

Cleaning and sterilisation of equipment is important as are the sources of bedding and feed.

Biosecurity

Some animals will be SPF and will have to be moved and held in appropriate isolators to prevent introduction of organisms from which they are free. There are important zoonoses which require high levels of security. Biosecurity levels have been described by Murray (1998) and are recorded in the International Animal Health Code of the OIE.

Systems of biosecurity have four levels. The OIE Code specifies:

- Group 1 animal pathogens: enzootic disease organisms. No official control.
- Group 2 animal pathogens: exotic or enzootic organisms. Low risk of spread. Official control. Not vectored, species specific, limited economic significance.
- Group 3 animal pathogens: exotic or enzootic organisms. Moderate risk of spread. Official control. May be vectored, quarantine applied, severe economic significance.
- Group 4 animal pathogens; Exotic or enzootic organisms. High risk of spread. Official control. May be vectored, quarantine applied, movement controls, severe economic significance.

Laboratories handling groups 3 and 4 operate at negative pressure, compared to the environment, have HEPA filtration of exhaust air and treatment of liquid and solid effluent to inactivate organisms. Operatives must shower out. Group 4 also requires full isolation in closed (class 3) biosafety cabinets or the use of full body suits.

Disease concerns

The principal diseases in laboratory mice and rats that are of concern to AQIS include:

Ectromelia (mousepox).

Ectromelia is a highly contagious poxvirus infection of laboratory mice. Infection of naive mice can result in 100% of animals of susceptible strains (eg. BALB/c, C3H) affected. Animals may die in the viraemic stage or develop generalised skin rash. Sub-clinical ectromelia infection may be converted to clinical disease by many common laboratory manipulations.

Lymphocytic choriomeningitis virus (LCM)

LCM is a natural infection of wild and laboratory mice and Syrian hamsters. Humans, monkeys, dogs, rabbits, guinea pigs, rats and chickens are susceptible to infection and it is because of its zoonotic potential that imported animals have to be free of infection. Clinical disease in mice is highly variable depending on the virus strain, mouse strain and age at infection. In humans infection can cause serious and fatal disease.

Hantaan virus infection.

Hantaan virus is the prototype virus of a group of viruses in the Hantavirus genus. Hantaan virus causes clinically inapparent infection in rats but severe disease (Korean haemorrhagic fever) in humans. Naturally infected laboratory rats have been the source of hantavirus infection in research workers in Japan, Belgium, UK and France.

Other agents that are of less concern to quarantine authorities but which should be of concern to importers are:

Murine parvoviruses (MVM, MPV)
Mouse hepatitis virus
Pneumonia virus of mice
Reovirus type 3
Sendai virus
Theiler's encephalomyelitis virus
Rat parvoviruses (KRV, Toolan's H-1, RPV)
Mouse adenovirus

Rat coronaviruses (RCV, SDAV)
Mouse polyoma virus
Rotavirus (EDIM)
Mouse cytomegalovirus
Mycoplasma pulmonis
CAR bacillus
Clostridium piliforme
Encephalitozoon cuniculi
Citobacter rodentium (freundii BT4280)
Salmonella spp
Dermatophytes (ringworm)
Internal nematodes (eg., pinworm)
Skin mites

Conclusion

The AQIS processes of establishment and regulation of importation requirements are designed to protect the community and the animals concerned. They are scientifically based and must meet national and international obligations. The processes are transparent and stakeholders and interested parties have an opportunity to participate and should do so in order to ensure the best means of achieving disease control objectives are employed. Modern methods of self regulation through quality assurance measures are encouraged.

References and further reading

- Aiello, S.E. and Mays, A., (eds.) (1998) *The Merck Veterinary Manual*. 8th Ed, Merck and Co, Whitehouse Station, New Jersey.
- AQIS (1991) *The Application of Risk Management in Agricultural Quarantine Import Assessment: a discussion paper*. Australian Government Publishing Service, Canberra.
- AQIS (1998) *The AQIS Risk Analysis Process Handbook*. Australian Quarantine and Inspection Service, Canberra.
- Cannon, R.M. and Roe, R.T.. (1982) *Livestock Disease Surveys. A Field Manual for Veterinarians*. Bureau of Rural Science, Department of Primary Industry. Australian Government Publishing Service
- Garner, M.G. and Nunn M.J. (1995). The Australian national animal health information system. *Epidemiol. sante anim.*, **27**: 143-160.
- Hueston W.D. (1993). Assessment of national systems for the surveillance and monitoring of animal health. *Rev. Sci. Tech. Off. Int. Epiz.*, **12 (4)**: 1187-1196.
- International Air Transport Association, Live Animals Regulation*, 15th Ed July, 1988.
- MacDiarmid, S.C. (1991). Risk analysis and the importation of animals. *Surveillanc* **18 (5)**: 8-11.
- MacDiarmid, S.C. (1993). Risk analysis and the importation of animals and animal products. *Rev. Sci. Tech. Off. Int. Epiz.* **12(4)**: 1093-1107.
- Morley, R.S. (1993). A model for the assessment of the animal disease risks associated with the importation of animals and animal products. In risk analysis, animal health and trade *Rev. Sci. Tech. Off. Int. Epiz.* **12 (4)**: 1055-1092.
- Murray, P.K. (1998) An overview of the roles and structure of the international high-security veterinary laboratories for infectious diseases. *Rev. Sci. Tech. Off. Int. Epiz.* **17 (2)**: 426-443.

Office International des Epizooties (OIE) (1994). *International Animal Health Code*, 1993 and 1994 updates, (6) 27-28/19.

Office International des Epizooties (OIE) (1992). *Manual of Standards for Diagnostic Tests and Vaccines*, 3rd Ed. OIE Paris 723 pp.

Nairn, M.E., Allen, P.G., Inglis, A.R. and Tanner, C. (1996) *Australian Quarantine: a shared responsibility*. Department of Primary Industries and Energy, Canberra.

World Trade Organisation (1994) *Agreement on the Application of Sanitary and Phytosanitary Measures*, WTO, Geneva.

Editor's Notes:

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