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## **ASSESSMENT OF QUALITY IN COMMERCIALLY SUPPLIED GENETICALLY MODIFIED ANIMALS**

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### **INTRODUCTION**

The use of genetically modified animals has increased significantly in recent years. In 2001 in the UK, 24% of all animals used in experiments were genetically modified<sup>1</sup>. In the same year in the Netherlands, 19% of all animals used in experimental procedures were genetically modified<sup>2</sup>.

These figures reflect a significant change in the use of animals in research in recent years. However, additional risks to the microbiological quality of experimental animals have also been introduced because of the increased movement of genetically modified animals between research facilities.

### **WHAT ARE THE CONCERNS?**

Institutional policies for the importation of animals have changed in recent years. Prior to the mid-1990s, animal facility managers were able to limit the sources of imported animals to a small number of commercial suppliers. In this way, the risks of importing unwanted organisms were minimized. However, genetically modified animals may originate from diverse worldwide sources, and the incidence of new infection has

increased<sup>3</sup>. This may result in animal welfare concerns due to outbreaks of disease and devastating effects on research due to changes in biochemical, physiological or other parameters. Below are a few examples of infections that may be introduced to animal facilities through the importation of transgenic animals

## INFECTIONS

### Mouse Hepatitis Virus (MHV)

MHV is a coronavirus and comprises a group of serologically and genetically related, but distinct, strains. About 25 strains have been reported. However, like other coronaviruses, MHV rapidly mutates and strains readily form recombinants. Therefore, the number of strains may possibly be larger. MHV is extremely contagious and is possibly the single most difficult agent to control in laboratory mice. In infected colonies most, if not all, weanling and older mice will be serologically positive. Coronaviruses are strongly immunomodulating, may interfere with oncology research, reproductive technology, may alter physiological parameters such as liver enzyme levels, patterns of protein synthesis and may interfere with experiments involving other infectious agents<sup>4</sup>. Because some transgenic animals may originate from facilities where MHV is endemic, the risk to the importing facility is obvious

### Pinworm

The most common pinworms in laboratory animals are *Syphacia* spp and *Aspiculuris tetraptera*. *Syphacia* spp has an 11-15 day life cycle; the life cycle of *A. tetraptera* is 23-25 days. The eggs are resistant in the environment and may remain viable for long periods. The latter characteristics make this organism particularly undesirable in animal facilities, for once it is established, it is difficult to eradicate. Eradication usually requires fumigation of the animal facility and may require rederivation of infected animals by hysterectomy or embryo transfer. However, treatments are available. Inclusion of fenbendazole in feed has been used with success<sup>5</sup>. Although pinworm is not considered a pathogen of laboratory animals, it is immunomodulating, may interfere with growth studies and has been shown to impact on behavioural experiments. Pinworms are frequent contaminants of imported transgenic animals<sup>4</sup>.

## **Pasteurella pneumotropica**

*P. pneumotropica* is a common bacterial organism, even in facilities where hygiene standards are high. In infected colonies, it can be isolated from up to 95% of the animals. It is generally not considered to be pathogenic. However, it may be of significance in immunocompromised hosts. It has also been isolated in cases of conjunctivitis, panophthalmitis, dacryoadenitis, subcutaneous and cervical abscesses, bulbourethral gland infections, uterine infections and otitis media<sup>4</sup>. Eradication of the organism is by hysterectomy or embryo transfer rederivation.

## **Helicobacter spp**

Various *Helicobacter* spp have been identified. *H. hepaticus* and *H. bilis* have been demonstrated to cause hepatic disease and lesions in mice. *H. muridarum* colonises the gastric mucosa and intestine of mice but is not associated with intestinal or hepatic disease. *H. rodentium*, also found in mice, causes lesions in immunocompromised mice but is not associated with any lesions in immunocompetent mice<sup>6</sup>.

## **Ectoparasites**

Over the last 20 years the incidence of ectoparasites in laboratory animal colonies decreased significantly as higher standards of hygiene and stricter controls have been introduced. However, there has been an recent increase in the incidence of these organisms, which has coincided with the movement of transgenic animals between diverse sources. They may cause pruritis, hairloss, scratch wounds and ulcerative pyodermatitis. In addition, they may interfere with research in a variety of ways, including inducing allergic reactions in mice. They may also serve as vectors for other infectious diseases<sup>4</sup>.

## **BIOSECURITY**

The real and current challenges for the animal facility manager are to identify, reduce, exclude and eliminate adventitious infectious agents from genetically modified animals, whilst maintaining their biological integrity<sup>7</sup>. Biosecurity is effected using physical restrictions to pathogen entry and putting into place procedures that will maximise the effect of the physical barrier. However, biosecurity is also a *culture* that is engendered within all

staff working with animals, where risks to the microbiological security of the animal facility are always given priority consideration. This particularly the case when reviewing health monitoring reports for animals to be imported, especially from non-commercial sources.

Such reports should always be requested from suppliers, be they commercial or non-commercial. Care must be exercised in reviewing these reports, as information may vary in format from facility to facility. The following is considered essential: the barrier from which the animals originate has been microbiologically screened within the last 3 months; the sample size was sufficient to provide a statistically valid result; the range of organisms and agents monitored conforms to that required by the importing facility. As a general guide, the FELASA Recommendations for health monitoring of breeding and experimental colonies should be followed.

It is generally recommended that, in any case, imported animals particularly those originating from non-commercial sources, be quarantined and tested for unwanted agents prior to entry to the facility<sup>8</sup>. Physical restrictions to pathogens may be: 1) barrier buildings, 2) flexible film isolators, and 3) Individually Ventilated Caging systems (IVCs)

Barrier buildings may vary according to the facility, but many will include a dedicated autoclave, air handling and filtration systems, positive air pressure, water treatment and filtration. There will also be facilities of varying severity for the entrance of personnel and materials into the facility. Flexible film isolators may either be positive or negative pressure. Positive pressure isolators are for the exclusion of unwanted organisms from the animals held within the isolator; negative pressure isolators will exclude unwanted organisms harboured by animals within the isolator from animals held in facilities outside the isolator<sup>9</sup>.

IVCs provide individual *microbiological entities* for small groups of animals. Because of the nature of IVCs, each cage is microbiologically separate from its neighbour. Therefore, animals of varying microbiological quality may be housed on the same rack. Such systems provide a challenge for health monitoring because of the difficulty in monitoring adequately each of the individual cages on the rack.

Procedures that may be introduced in order to minimise the risk of ingress of unwanted agents include: 1) quarantine of incoming animals prior to introduction to the facility, 2) control of the introduction of biological materials and 3) restriction of personnel movement.

No animal should be permitted to enter the facility without authorisation. Preferably, new animals should be received into an animal receiving area. The personnel in that area can then identify the correct housing conditions, ensure that quarantine procedures are followed. In so-called 'closed' facilities, new animals may only enter the facility following caesarian or

embryo rederivation. In order to ensure that the quarantine area itself does not pose risks to the rest of the facility, it should preferably operate on an ‘all-in-all-out’ basis, so that it can be thoroughly cleaned and disinfected/sterilized between shipments<sup>10</sup>.

Only authorized personnel should be permitted into the facility. It is highly desirable that a policy of quarantine for personnel is applied to ensure that there is a period of absence from contact with other animals prior to entry to the facility. Protective clothing and showers should be available. Once policies for personnel have been established and agreed, then it is vital that they are followed rigidly<sup>9,10</sup>.

Biological products represent a serious risk to research animal facilities. Nicklas<sup>11</sup> demonstrated that a significant percentage of transplantable tumors and rodent cell lines are contaminated by a variety of agents. Therefore, a policy for the safe introduction of biological materials into the facility should be established. This should include testing of cell lines before they may be considered for acceptance. This is conventionally done using a Mouse (Rat or Hamster) Antibody Production Test M(R,H)AP test. Other tests are now available, for example the IMPACT test using molecular biological techniques, which provide faster results without the need for experimental animals.

## **Background Genetic Information**

Genetically modified animals are generally imported on the understanding that they bear the genetic modification expected. However, this may not always be the case, and it is worthwhile to obtain from the supplying facility details of what animals are being sent, together with supporting information on genetic background, relating to the genetic modification, and the background strain. For example, the 129 inbred mouse has been the most widely used strain in the production of targeted mutations due to the availability of several lines of embryonic stem cells. However, there is substantial genetic variation among sub strains of the 129 strain and the choice of a particular one may be of critical importance for a particular project<sup>12</sup>.

The genetic status of the background strain is significant. Therefore, continued checks on the colony providing this strain are important to ensure that no genetic contamination has occurred, which may later confound the interpretation of experimental data.

## Continued Testing

The following continued checks are recommended for animals within the facility and for animals on experiment: 1) routine health monitoring based on FELASA Recommendations. 2) testing the continued genetic authenticity of inbred strains held in the facility used in backcrossing and 3) Testing for the presence of the transgene in the animals used in a research project, and in other transgenic animals held in the same room.

## SUMMARY

- The increased movement of genetically modified animals has resulted in an increase in the incidence of microbiological contamination of laboratory animal facilities.
- Many of these contaminants may have significant effects on the outcome of experiments
- Institutional policies for the introduction of animals from outside sources should be established and followed.
- Attention should be paid to the correct and rigorous operation of the barrier system.
- Genetic authenticity of transgenic and background strains should be assured.
- Continuing checks on the health and genetic status of genetically modified animals is recommended.

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