

Rodent and Germplasm Trafficking: Risks of Microbial Contamination in a High-Tech Biomedical World

Esther Mahabir, Beth Bauer, and Jörg Schmidt

Abstract

High-tech biomedical advances have led to increases both in the number of mice used for research and in exchanges of mice and/or their tissues between institutions. The latter are associated with the risk of dissemination of infectious agents. Because of the lack of international standardization of health surveillance programs, health certificates for imported rodents may be informative but may not address the needs of the importing facility. Preservation of mouse germplasm is achieved by cryopreservation of spermatozoa, embryos, or ovaries, and embryonic stem cells are used for the production of genetically engineered mice. After embryo transfer, recipients and rederived pups that test negative in microbiological screening for relevant microorganisms are released into full barrier holding areas. However, current research shows that embryos may also transmit microorganisms, especially viruses, to the recipient mice. In this article, we discuss regulations and practical issues in the shipping of live mice and mouse tissues, including spermatozoa, embryos, ovaries, and embryonic stem cells, and review work on microbial contamination of these biological materials. In addition, we present ways to reduce the risk of transmission of pathogens to mice under routine conditions.

Key Words: assisted reproductive technologies; embryos; germplasm; mice; microbial contamination; pathogens

Prevalence and Persistence of Pathogens in Mice

Knowledge of the prevalence, replication, and persistence of pathogens in a mouse facility aids both in assessments of the risk of transmission to other mice

Esther Mahabir, PhD, is head of research and development in Reproductive Biology and Microbiology, head of the Mouse Core Unit, and head of CryoGene Services – Embryo Cryopreservation and Rederivation in the Department of Comparative Medicine at the Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH) in Neuherberg, Germany. Beth A. Bauer, DVM, is a clinical assistant professor at the Research Animal Diagnostic Laboratory (RADIL), Rat Resource and Research Center (RRRC), and Mutant Mouse Regional Resource Centers (MMRRC) at the University of Missouri, Columbia. Jörg Schmidt, DVM, is a professor in and head of the Department of Comparative Medicine at the German Research Center for Environmental Health (GmbH) in Neuherberg.

Address correspondence and reprint requests to Dr. Esther Mahabir, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstrasse 1, D-85764 Neuherberg, Germany or email mahabir@helmholtz-muenchen.de.

and mouse facilities and in the development of suitable management strategies to eliminate such risks.

But there is a lack of current, comprehensive information on the prevalence of bacteria and parasites in mice in Europe (for detailed information on US prevalence of these and murine viruses see Carty 2008). With respect to murine viruses, a summary of data from the past decade shows that the most prevalent in the United States (Carty 2008; Livingston and Riley 2003) and Europe (Schoondermark-van de Ven et al. 2006) are mouse hepatitis virus (MHV¹), parvoviruses, mouse rotavirus, Theiler's murine encephalomyelitis virus (TMEV¹), reovirus type 3 (reo 3), Sendai virus, and mouse adenovirus (MAcV) (FL + K87). The report on European prevalence (Schoondermark-van de Ven et al. 2006) showed that the main viruses of concern there are MHV (12%), parvoviruses (8.8%), mouse rotavirus (3.7%), TMEV (2.2%), reo 3 (0.6%), pneumonia virus of mice (PVM, 0.2%), Sendai virus (0.2%), and polyoma (0.1%). The same report noted that viruses such as lymphocytic choriomeningitis virus (LCMV), mouse K virus, ectromelia virus, MAcV, and mouse thymic virus (MTV) have not been detected in Europe in the last decade. These data are based on serological analysis of 80% of samples obtained between 2000 and 2003 from more than 100 different institutions including universities, research centers, breeding companies, and industry in the Netherlands (45%), France (35%), Belgium (7%), and other European countries (13%, mainly Germany and Switzerland).

Since the discovery of murine norovirus (MNV¹) in 2003 (Karst et al. 2003), its prevalence is reported to be 22.1% from 12,639 mouse serum samples collected in the United States and Canada in 2003 (Hsu et al. 2005). Perdue and colleagues (2007) reported a prevalence ranging from 2% to 83% in sentinel mice from five US mouse facilities. In some German mouse facilities, MNV had a prevalence of 60% (Nicklas et al. 2006), 64.3% (Müller et al. 2007), and 0% to 69%, depending on the type of mouse holding area (Mahabir et al. 2007b).

Some viruses, such as MHV, mouse rotavirus, and Sendai virus, are shed for only a short period of time, whereas ectromelia virus and PVM are shed for a moderate length of time, and other viruses such as LCMV, lactate dehydroge-

¹Abbreviations used in this article: ES, embryonic stem; ET, embryo transfer; IVC, individually ventilated cage; IVF, in vitro fertilization; MHV, mouse hepatitis virus; MVM, minute virus of mice; MNV, murine norovirus; MPV, mouse parvovirus; TMEV, Theiler's murine encephalomyelitis virus; ZP, zona pellucida

nase-elevating virus (LDHV), murine cytomegalovirus (MCMV), minute virus of mice (MVM¹), mouse parvovirus (MPV¹), TMEV, and MTV persist for longer periods of time (Compton and Riley 2001). A comprehensive list of viruses and their target tissues is available in Compton and Riley (2001).

Shipping of Live Mice and Cryopreserved Materials

Transportation of live mice and mouse cells and tissues from one institution to another is crucial to biomedical research in the international community. For the benefit of the mice, it is important to thoroughly plan such transportation before making shipping decisions. Major proactive points of consideration include the observance of national legislation of all aspects of genetic technology and biosafety, international shipping regulations, packaging requirements, and consultations with and requirements of the exporting and importing institutions.

Shipment of Live Mice

US guidelines have recently been published for the transportation of laboratory animals (NRC 2006), and European guidelines are summarized in Appendix A of the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (ETS No. 123; <http://conventions.coe.int>). The UK Laboratory Animal Science Association (LASA) also has published *Guidance on the Transport of Laboratory Animals* (Swallow et al. 2005).

For shipments of live mice, specialized courier companies provide professional and comprehensive services. They are experienced in global logistics as well as country- and species-specific requirements and regulatory documents and concerns. In addition to importing/exporting country requirements, the International Air Transport Association (IATA) regulations should be followed when packing and preparing live animals for air transport (IATA 2007). Packages for any kind of animal transport should be designed to prevent the animals' escape, exclude the entry of microorganisms, allow visual inspection of the animals without compromising their microbiological status, and allow external disinfection of the package on arrival at the receiving facility (Lee et al. 2007). In addition, the following measures are indispensable to ensure minimal transit time and facilitate the animals' arrival in good health at their final destination:

- a recent health report by a veterinarian of the exporting institution's animal colony including a 1½-year history of the colony's health status,
- packaging in adequate transport boxes, with bedding, food, and water,

- safety and stress reduction measures for the animals, and
- early contact with the shipping corporation or carrier.

For regional shipping, ground transport may pose the least stress as it entails minimal handling of the shipping container and a single environment during transportation. Ground transportation should be carried out by licensed personnel in climate-controlled vehicles using the shortest routes. Regional shipments by ground transportation normally involve one licensed carrier, whereas long distance or transcontinental shipping generally requires coordination of both ground transport and air freight and may be performed by multiple subcontractors, resulting in longer transit times and more handling, both of which may be more stressful for the mice (NRC 2006).

For the animals' comfort, it is particularly important to ensure suitable environmental conditions throughout the shipping. Further to the stress involved in any shipment, longer journeys (particularly international or transcontinental journeys) also affect their diurnal rhythm. Mice that experience shifts in light/dark cycles require up to 2 weeks to normalize (Weinert et al. 1994) and thus require a longer period of overall adaptation and restoration, ranging from 1 to 7 days to several weeks or even months, depending on the stress to which they were exposed during shipment (Obernier and Baldwin 2006).

The US Department of Agriculture (USDA) guidelines for the importation of live laboratory animals set forth regulations on international transport of laboratory mice and their tissue to the United States (<http://www.aphis.usda.gov/vs/ncie/ilive-mam.html>). Other US regulatory agencies that may have oversight of research animal importation or shipment are the Centers for Disease Control and Prevention (CDC), the Department of Transportation (DOT), and the US Fish and Wildlife Service. In general, research mice are exempt from these regulations provided that they are not carrying infectious diseases and are from the genus *Mus musculus*. Imports to countries of the European Union (EU) require the permission of the local government.

To ensure US customs clearance, the following documentation should accompany the shipment: a pro forma invoice stating that the shipment contains "Live Laboratory Mice" and listing the species (*Mus musculus*), number, gender, age, type of package, and names and addresses of the exporting and importing institutions. The invoice should also include a statement that the animals do not meet the criteria for an endangered species nor pose a risk to human health. In addition, a health certificate is required with, if appropriate, a veterinarian's statement that "Animals are healthy and have not been exposed to or inoculated with any livestock or poultry disease agents exotic to the United States" and that "The animals have not originated from a facility where work with exotic disease agents affecting livestock or poultry is conducted."

Some countries may have additional exportation documents that must be completed before the animals leave the

country of origin, and commercial airlines also may ask for additional documents, which should be applied for and prepared well ahead of the time of shipment. For live mice and the cells and tissues derived from them some institutions require material transfer agreements (MTAs) or, if the material is transferred on a collaborative basis, a short plan of the research project before shipment. Exportation of live mice from the United States or the European Union is not regulated but the shipment must conform to IATA regulations and the destination country's regulations.

Because each country has its own regulations and requirements it is highly advisable to use a customs broker and/or a courier company knowledgeable in live animal shipping for international shipments of live mice.

Shipment of Cryopreserved Materials

Cryopreservation of embryos (Glenister et al. 1990; Shaw and Nagakata 2002) and spermatozoa has become a routine mechanism to preserve mouse models and to transfer single mice or colonies without the welfare concerns that arise in the shipping of live animals. Additional advantages to shipping cryopreserved germplasm (spermatozoa, oocytes, and resulting embryos) are the suitability of cryopreserved materials for storage and use when needed and for preliminary testing of sample aliquots to determine microbiological status before use.

Many institutions (including both of ours) have established transgenic animal cores that can assist in the recovery of cryopreserved materials. But if this expertise is not available at an investigator's institution there are established regional repositories in countries around the world that can assist in the recovery of cryopreserved materials; a number of these repositories have joined together to form the Federation of International Mouse Resources (FIMRe; Table 1). Many members are working together to assist the transfer of models across continents from one repository to another to facilitate their use by individual investigators (Davisson 2006). These cooperative agreements will enable investigators to obtain recovered live mouse models from their local regional repository even if the model is cryopreserved and held in another country.

Cryopreserved oocytes, embryos, spermatozoa, and ovaries are typically shipped in a "dry shipper" in which the liquid nitrogen is absorbed in the shipper liner. These containers usually maintain an ultralow temperature for 7 to 21 days, depending on the model of shipper. In compliance with IATA regulations, documentation of an inversion test should be included to demonstrate that no free liquid nitrogen is present in the shipper. Cell lines, including murine embryonic stem (ES¹) cells, and tissues are usually shipped on dry ice, which is considered a hazardous material and has special packaging and labeling requirements (set forth in the IATA regulations). Alternatively, for short-term (less than

Table 1 Federation of International Mouse Resources (FIMRe) Member Mouse Resources by Continent

Resource Center	Location	Website
North America		
American Type Culture Collection (ATCC)	Virginia, USA	www.atcc.org
Canadian Mouse Mutant Repository (CMMR)	Toronto, Canada	www.cmmr.ca
Canadian Mouse Consortium	Canada	www.mousecanada.ca
The Jackson Laboratory	Maine, USA	www.jax.org
Mouse Models of Human Cancers Consortium (MMHCC)	Maryland, USA	emice.nci.nih.gov; mouse.ncifcrf.gov
Mutant Mouse Regional Resource Centers (MMRRC)		www.mmrc.org
University of Missouri/Harlan Consortium	Missouri, USA	www.mmrc.missouri.edu
University of California, Davis	California, USA	ccm.ucdavis.edu/mmrc
University of North Carolina at Chapel Hill	North Carolina, USA	www.med.unc.edu/mmrc
Europe		
The European Mouse Mutant Archive (EMMA)		www.emmanet.org
CNR-IBC—Consiglio Nazionale delle Ricerche		
Istituto di Biologia Cellulare	Monterotondo, Italy	www.emma.cnr.it/CNR-IBC.html
CNRS—Institut Transgenose	Orleans, France	transgenose.cnrs-orleans.fr
FCG—Instituto Gulbenkian de Ciência	Oeiras, Portugal	www.igc.gulbenkian.pt
GSF—Institute of Experimental Genetics	Neuherberg, Germany	www.gsf.de/ieg
KI—Karolinska Institutet	Stockholm, Sweden	ki.se/cmb
MRC—Mammalian Genetics Unit	Harwell, UK	www.har.mrc.ac.uk
Asia		
RIKEN BioResource Center (RBRC), Experimental Animal Center	Tsukuba, Japan	www.brc.riken.jp/lab/animal/en
Center for Animal Resources and Development (CARD)	Kumamoto, Japan	card.medic.kumamoto-u.ac.jp/card/english
Australia		
Australian Phenomics Facility	Canberra, Australia	www.apf.edu.au

24 hours) shipping, embryos can be transported in holding medium in cryopreservation straws at room temperature. Likewise, murine ES cells can be sent directly in the culture flask at room temperature.

The importation paperwork for cryopreserved laboratory mouse tissues and cell lines is similar to that required for live animal importation to the United States (i.e., a pro forma invoice and declaration statements). In addition, any cells or tissues grown *in vitro* before shipment must be declared free of animal cell culture-derived products of livestock origin, particularly fetal or adult bovine serum. Shipments of cell lines or tissues that contain a material of livestock origin such as fetal bovine serum require a USDA importation permit.

Above all, proactive communication between the exporting institution, carrier, veterinary and legal authorities, regulatory agencies, and importing institution is of paramount importance for safe and successful deliveries.

Microbial Contamination of Germplasm

The oocyte and preimplantation embryo are surrounded by a zona pellucida (ZP¹), essentially a sulphated glycoprotein gel of the order of 2% to 6% (weight/volume) (Green 1997) that protects the embryo from its environment (Epifano and Dean 1994; Wasserman et al. 1996). After removal of the surrounding cumulus cells, the ZP is composed of a complex fibrous network interspersed with numerous pores that are largest at the outer surface and decrease in size centripetally.

In viremic mice the reproductive tract may be susceptible to infectious pathogens either locally or systemically and viruses can spread from there to various tissues and organs. In mouse germplasm cells, pathogens may (1) be present in the oocyte at fertilization and replicate in the embryo, fetus, or pups; (2) be present in or attached to the spermatozoa and carried into the oocyte at fertilization; (3) traverse, become embedded in, or adhere to the ZP; or (4) be present in the embryo after damage to the ZP during handling or manipulation such as assisted reproductive technologies (ARTs). Oocytes and embryos with an intact ZP subjected to vigorous pipetting may rupture, and are further at risk when the ZP is partially disrupted from ARTs such as intracytoplasmic sperm injection (ICSI), subzonal injection (SUZI), microinjection of oocytes, and blastocyst injection.

There are typically four main experimental designs to determine the risk of pathogen transmission by *in vivo*-derived embryos (Hare 1990):

- *in vitro*-*in vitro*: embryos from clean donors are exposed to the pathogen *in vitro* and assayed *in vitro*
- *in vitro*-*in vivo*: embryos from clean donors are exposed to the pathogen *in vitro* and transferred to seronegative recipients, which are screened for the development of antibodies to the pathogen

- *in vivo*-*in vitro*: embryos are collected from infected and/or seropositive donors and assayed *in vitro*
- *in vivo*-*in vivo*: embryos are collected from infected and/or seropositive donors and transferred to seronegative recipients, which are screened for the development of antibodies to the pathogen.

In efforts to gain more knowledge about the epidemiological potential of current and emerging ARTs, the main difficulty of working with an *in vivo* system is the selection of mice that are carrying the virus as they may be seropositive but not necessarily virus positive. Many of the well-known murine pathogens are shed for a short period of time and diagnosis of virus-positive mice depends heavily on environmental monitoring. In some cases, fecal samples are adequate for antemortem determination of the microbiological status (e.g., for parvoviruses, MNV, MHV, mouse rotavirus, reo 3, and TMEV). In order to gain as much information as possible about the biological interaction of the germplasm with pathogens it is generally speedier to perform experimental work that more or less simulates *in vivo* conditions. However, experimental designs often include infections with high viral doses to demonstrate the “worst case” scenario.

In the *in vitro* system, pathogens may be present either in the collecting media or in or on the gametes or embryos themselves. *In vitro* fertilization (IVF¹) with infected spermatozoa and/or oocytes is an effective method to determine the risks posed by gametes or *in vitro*-derived embryos. The resulting embryos are analyzed *in vitro* by polymerase chain reaction (PCR) and/or are transferred to seronegative recipients that are monitored for seroconversion. It is also useful to screen their pups for the presence of the virus by PCR or serological analysis. A further step in determining the epidemiological potential of such germplasm is to monitor the collecting and washing fluids for the presence of the infectious agent, usually by PCR and virus isolation in cell culture. Embryos are usually kept in short-term culture (e.g., for 1 day) to prevent low levels of viruses from replicating to such an extent that they pose a real threat.

Transmission of Pathogens by Spermatozoa

IVF with fresh samples of mouse spermatozoa resulted in live offspring as early as 1969 (Mukherjee and Cohen 1970), and cryopreservation of sperm with subsequent IVF and recovery of live mice started in the early 1990s (Yokoyama et al. 1990). Advances in cryopreservation of spermatozoa, IVF, ICSI, and embryo culture techniques make sperm cryopreservation a reliable means for preserving mouse strains and lines.

Few studies have examined the possibility of transmission of murine pathogens by spermatozoa. Dutko and Oldstone (1979) found that murine cytomegalovirus (MCMV) replicated in germ cells of the testes. Baskar and colleagues (1986) confirmed this observation and found viral particles

in spermatids; however, they did not detect any decreased fertility in these male mice and embryos developed normally to the blastocyst stage. Tebourbi and colleagues (2002) examined whether MCMV microinjected directly into zygote-stage embryos (simulating ICSI conditions) would result in infected live pups; they were able to infect the embryos, and the virus was present up until the blastocyst stage but disappeared in fetuses and pups. Retroviruses have been demonstrated to replicate in epididymal sperm epithelium and associate with spermatozoa as they move through the epididymis, suggesting that venereal transmission is possible (Kiessling et al. 1989).

Recent studies have focused on murine pathogens that are prevalent in mouse colonies. Scavizzi and Raspa (2004, 2006) examined testes and epididymis for the presence of *Helicobacter typhlonius* and MHV, both of which were present transiently. In these two studies, spermatozoa collected from males with testes that tested positive for either *H. typhlonius* or MHV failed to produce positive live pups after IVF and embryo transfer (ET¹). Peters and colleagues (2006) demonstrated that standard washing procedures in the IVF and embryo culture system are sufficient to eliminate MHV transmission when spermatozoa and oocytes are incubated with high levels of virus. MPV was recently found in spermatozoal samples (Agca et al. 2007), but it remains to be determined if embryos generated using MPV-contaminated sperm result in infected offspring. In a recent study (Mahabir et al. 2007a) we demonstrated that MVM-exposed *in vivo*-derived embryos washed ten times are still capable of transmitting the virus to recipient female mice. However, we also demonstrated that the IVF-ET procedure with MVM-contaminated spermatozoa resulted in the production of virus-free seronegative pups (Mahabir et al. 2008).

Additional studies are needed to determine whether other prevalent viruses such as MNV, rotavirus, or TMEV can be transmitted by spermatozoa. Methods to remove pathogens from mouse spermatozoa have not been widely explored but one study did demonstrate that percoll separation of spermatozoa was not sufficient to remove MPV (Agca et al. 2007).

Transmission of Pathogens by Ovaries, Oocytes, and Embryos

Pathogens can be transmitted by ET to recipients by contaminated personnel, instruments, or equipment, and transport or wash medium, as well as in or on the embryo itself, although an intact ZP is usually regarded as a mechanism for preventing pathogen transmission during ET. Agents that infect embryos could be viruses, bacteria, fungi, mycoplasmas, or parasites, but because of their size viruses are the most likely pathogens to be transmitted during ET.

Oocytes are typically collected and immediately used in ICSI or IVF with cryopreserved sperm, although cryopreservation of oocytes is possible and becoming more common

(Endoh et al. 2007). Females are superovulated and the cumulus oocyte complexes (COCs) can either be used as intact clutches for IVF or treated with hyaluronidase to remove the cumulus cells for ICSI. Wild-type oocytes are usually collected from female donors held in known pathogen-free colonies and are coincubated with spermatozoa containing the mutation or gene of interest. However, in rare instances, oocytes or ovarian tissue (Gunaseena et al. 1997; Hani et al. 2006) may be used as the primary means to recover mutant or transgenic mouse lines and the risk posed by pathogens may be higher in these cases.

Ovarian tissue cryopreservation is particularly useful for mouse models in which male infertility occurs (such as the X-autosome translocation mouse lines) or for rescue of lines in which only female animals are available (Disteche et al. 1979; Takahashi et al. 2001). Ovarian tissue transplantation carries an increased risk compared to gametes and embryos because it is a complex organ with multiple cell types, and tissue from viremic donors is likely to transfer infectious organisms. Retroviral contamination of donor tissue by a recipient female has occurred with transmission to recovered offspring (Lock et al. 1988), demonstrating that genetic alteration via retroviruses can occur in offspring as a consequence of either the donor or recipient female. Ovarian tissue from mice naturally infected with MHV has been shown to transmit MHV to the recipient females and offspring (Scavizzi and Raspa 2004). In contrast, *H. typhlonius*-infected ovarian tissue transplantation did not result in infection of the recipient female or offspring (Scavizzi and Raspa 2006). Researchers have found MPV in both ovarian tissue and oocytes (Agca et al. 2007), but it is unknown whether transplantation of infected ovarian tissue results in infection of recipient females and their offspring.

Very small viruses belonging to the *Picornaviridae* family—for example, Mengo virus, which is 27 to 28 nm (Gwatkin 1963, 1966, 1967), and the Coxsackie B-4 virus, which is 30 nm (Heggie and Gaddis 1979)—have been shown to traverse the ZP of murine embryos. Investigators have documented LCMV in mouse oocytes and embryos, providing evidence of transmission via the oocyte (Mims 1966), and Sendai virus (100 to 200 nm) in the murine ZP (Lavilla-Apelo et al. 1991, 1992; Tuffrey et al. 1972). One study reported infection with polyoma in the trophectoderm but not in the inner cell mass of murine embryos (Abramczuck et al. 1978).

Transmission of Pathogens by ES Cells

We include ES cells in this overview as they are fundamental to the production of genetically engineered mice. During blastocyst injection, besides feeder cells and the blastocysts themselves, the ES cells may harbor infectious pathogens. The exchange of ES cells between laboratories worldwide may increase the risk of transmitting mouse infectious agents as ES cells are often not screened for viruses, and viral contamination and infection are not readily detectable by cell morphology.

Although a survey of 46 ES cell lines did not show the presence of murine infectious agents (Nicklas and Weiss 2000), two studies showed that murine ES cells infected with MHV-2 (Kyuwa 1997; Okumura et al. 1996) and MHV-A59 (Kyuwa 1997) grew *in vitro* without showing either cytopathic effects or signs of differentiation (Kyuwa et al. 1997; Okumura et al. 1996). In addition to MHV, parvovirus and mycoplasma contamination has been found in ES cells submitted to diagnostic laboratories for PCR testing (BB personal communication with Lela Riley, University of Kansas, August 2007). The risk of ES cell contamination by feeder cells and embryos can be reduced by obtaining them from mice that are free of relevant pathogens.

Efficacy of Washing Protocols and Embryo Transfer in Eliminating Viruses

Washing protocols for embryos remove the viruses from the germplasm to a level below an infectious and/or immunogenic dose. Although there are well-established washing protocols for the embryos of some domestic animals such as cattle and pigs (Stringfellow and Seidel 1998), none exist for murine embryos. Some major contributing factors to reducing the risk of pathogen transmission during washing of the embryos include working under sterile conditions, repeated testing and use of pathogen-free reagents, use of a new pipette between washing drops, dilution of the washing fluids in a 1:100 range, and removal of ZP-less or damaged embryos before transfer to suitable recipient mice.

Because of the ZP's porous nature and depending on the virus size, even after washing there may be a risk of viruses nesting in the ZP micropores. For example, reports show that trysin treatment of embryos has not been successful in removing Sendai virus even after 12 washings (Lavilla-Apelo et al. 1991). Experimental infection with MVM and washing of embryos 10 times with medium revealed transmission of the virus by *in vivo*-derived embryos (Mahabir et al. 2007a) but not by embryos produced by IVF of oocytes with MVM-coincubated spermatozoa (Mahabir et al. 2008). This was an unexpected result that may be due to differences in the characteristics of the embryos themselves.

With respect to *in vitro*-derived embryos, the cumulus cells surrounding the oocytes may have adsorbed some of the virus thereby reducing the quantity left for entrapment in the micropores. Furthermore, the cumulus cells block entry of the virus as micropores are present only after removal of these cells. Removal of the cumulus cells and washing of oocytes have been shown to eliminate MPV contamination in mouse oocytes (Agca et al. 2007), and this approach should be sufficient to remove the majority of viral contaminants when they are not present in the oocyte itself.

Embryo transfer recipients in rederivation programs should be held in individually ventilated cages (IVCs¹) until testing shows that they are free of all unwanted microorganisms, including those listed in Appendix 3 of the Federation of Laboratory Animal Science Associations

(FELASA) recommendations (Nicklas et al. 2002). There should be a minimum of 6 weeks between the ET and microbiological examination of the recipients, coinciding with weaning of the offspring at 3 or 4 weeks of age (wild-type littermate pups should not undergo health monitoring until they are at least 6 weeks old).

Some mouse facilities screen recipients before releasing pups into full barrier areas and, for subsequent screening of the pups themselves, use sentinel mice or random samples from the colony. Other mouse facilities screen the recipient mother and at least one pup from each litter (usually a wild-type pup) before releasing rederived pups into full barrier areas. If wild-type pups are not available for screening, it may be preferable to use cohoused wild-type sentinels placed at the time of weaning rather than relying on test results of genetically altered mice whose immune status is usually unknown.

A number of reports have shown that *in vitro*-derived 2-cell embryos (Peters et al. 2006) and *in vivo*-derived 1-cell (Van Keuren and Saunders 2004) and 2-cell embryos (Carthew et al. 1983, 1985; Mahabir et al. 2007a; Reetz et al. 1988; Suzuki et al. 1996) did not pose a risk of transferring MHV during embryo transfer. In addition, embryos from mice from holding areas that were positive for MAdV, mouse rotavirus, MPV, or TMEV led to rederivation of the mouse lines (Van Keuren and Saunders 2004). The efficacy of embryo transfer for rederiving mice is reported to be high. Due to short periods of virus shedding, donor mice may be seropositive but may not be virus carriers, thus leading to the production of virus-free seronegative pups.

Reducing the Risks Posed by Mouse and Germplasm Trafficking

Laboratories that deal with frequent imports of mice and biological materials should establish and implement effective measures to reduce the risk of transmitting infectious agents to staff or existing animal colonies at the importing institution. Even collection of germplasm under sterile conditions does not prevent dissemination of infectious agents from colonies where they are prevalent. Although health certificates that precede or accompany an import colony provide information on the animals' health status and a 1- to 1½-year history of health data, because of the interval between collection and analysis of the samples and preparation of the health report, both mice and biological materials should be considered carriers of microorganisms unless analyses prove the absence of contaminants. Imported mice should therefore be kept in quarantine pending reevaluation of their health status and biological materials should be carefully screened before use.

To enable facility management to obtain sound information on imported animals' current health status at any given time, each facility should design and implement health monitoring programs based on its needs. These programs use randomly sampled mice, contact sentinels, or

sentinels exposed to soiled bedding. Some protocols also include monitoring of exhaust air for pathogens not transferred by soiled bedding (Brielmeier et al. 2006; Compton et al. 2004).

In recent years, there has been an increase in the use of IVC rack systems in laboratory rodent facilities. Recent data (Brielmeier et al. 2006) show that IVCs provide complete biocontainment when infected and noninfected mice are kept in separate cages in the same IVC rack provided that cage bedding is changed in class II laminar flow hoods or cage changing cabinets (CCCs) and appropriate standard operating procedures (SOPs) are strictly observed. In a typical IVC rack, each cage receives high-efficiency particulate air (HEPA)-filtered air. Mice are kept under positive pressure to protect them from airborne infectious or other noxious particulate agents in the environment (Clough et al. 1995; Cunliffe-Beamer and Les 1983; Lipman et al. 1993; Lipman 1999). Similarly, the exhaust air from the cages is HEPA-filtered before being returned to the room environment or to the heating, ventilation, and air conditioning (HVAC) system.

Maintaining mice under negative pressure prevents dissemination of pathogens into the environment and minimizes (Gordon et al. 2001; Lipman 1999) or prevents transfer of infectious agents from cage to cage in an IVC rack or room, depending on the IVC rack model. For biocontainment purposes, IVCs are optimal for holding imported mouse colonies under quarantine pending the completion of adequate microbiological analyses. Irrespective of the health report from the exporting institution, some facilities conduct routine prophylactic treatments of all imported and quarantined mice for pinworm and ectoparasites. Others refuse import applications for mice infected with parasites and authorize such imports only if the mice have been successfully treated at the exporting institution before shipment.

Once the health status of an imported mouse colony is found compatible with that of the importing institution mice may be transferred from the quarantine area to an experimental unit and released for investigators' use. Although IVCs meet a number of requirements for breeding and holding mice under specific pathogen-free (SPF) conditions in experimental units, filter-top cages are an economical and suitable alternative. For core breeding units and long-term holding of laboratory mice under SPF conditions, full barrier areas with a wet entry system or air shower and the use of IVCs have become an international standard. In general, these units do not allow access to investigators and mice are imported into the barrier only by ET.

Trafficking of biological materials, including germplasm and ES cells, should meet the same hygienic standards as those required for importing laboratory mice. Care should be taken to ensure that collection and/or processing of cells and tissues of the germplasm are performed under sterile conditions. To determine microbiological status—for example, if investigators suspect the presence of a particular agent or wish to screen for a limited number of agents—it

may be useful to test aliquots of biological materials, collection media, or washing drops by molecular biological analysis. Well-established sensitive PCR techniques are available for determining the microbiological status of murine biological materials in-house or by commercial laboratories. In addition, the mouse antibody production (MAP) test (Bauer et al. 2004; Blank et al. 2004; Bootz et al. 2003; Livingston et al. 2004; Mahabir et al. 2004) is also performed as antibodies to several viruses are detectable by standard serological analyses. For such tests, mice should be free of all FELASA-listed microorganisms (as well as MNV) and held under conditions that prevent dissemination of infectious agents.

Conclusion

Taken together, health monitoring, evaluation of the risk of pathogen transmission by mouse and germplasm trafficking, and appropriate managerial strategies are fundamental components of a facility's quality assurance program. Last but not least, the establishment and implementation of suitable quality assurance programs are based on the expertise of the facility's supervisory staff, who must have sufficient knowledge of and experience in laboratory animal science (Nevalainen et al. 1999).

References

- Abramczuk J, Vorbrodt A, Solter D, Koprowski H. 1978. Infection of mouse preimplantation embryos with simian virus 40 and polyoma virus. *Proc Natl Acad Sci U S A* 75:999-1003.
- Agca Y, Bauer BA, Johnson DK, Critser JK, Riley LK. 2007. Detection of mouse parvovirus in *Mus musculus* gametes, embryos, and ovarian tissues by polymerase chain reaction assay. *Comp Med* 57:51-56.
- Baskar JF, Stanat SC, Huang ES. 1986. Murine cytomegalovirus infection of mouse testes. *J Virol* 57:1149-1154.
- Bauer BA, Besch-Williford CL, Riley LK. 2004. Comparison of the mouse antibody production (MAP) assay and polymerase chain reaction (PCR) assays for the detection of viral contaminants. *Biologicals* 32: 177-182.
- Blank WA, Henderson KS, White LA. 2004. Virus PCR assay panels: An alternative to the mouse antibody production test. *Lab Anim (NY)* 33:26-32.
- Bootz F, Sieber I, Popovic D, Tischhauser M, Homberger FR. 2003. Comparison of the sensitivity of in vivo antibody production tests with in vitro PCR-based methods to detect infectious contamination of biological materials. *Lab Anim* 37:341-351.
- Brielmeier M, Mahabir E, Needham JR, Lengger C, Wilhelm P, Schmidt J. 2006. Microbiological monitoring of laboratory mice and biocontainment in individually ventilated cages: A field study. *Lab Anim* 40:247-260.
- Carthew P, Wood MJ, Kirby C. 1983. Elimination of Sendai (parainfluenza type 1) virus infection from mice by embryo transfer. *J Reprod Fertil* 69:253-257.
- Carthew P, Wood MJ, Kirby C. 1985. Pathogenicity of mouse hepatitis virus for preimplantation mouse embryos. *J Reprod Fertil* 73:207-213.
- Carty AJ. 2008. Opportunistic infections of mice and rats: Jacoby and Lindsey revisited. *ILAR J* 49:272-276.
- Clough G, Wallace J, Gamble MR, Merryweather ER, Bailey E. 1995. A positive, individually ventilated caging system: A local barrier system to protect both animals and personnel. *Lab Anim* 29:139-151.

- Compton SR, Riley LK. 2001. Detection of infectious agents in laboratory rodents: Traditional and molecular techniques. *Comp Med* 51:113-119.
- Compton SR, Homberger FR, Paturzo FX, Clark JM. 2004. Efficacy of three microbiological monitoring methods in a ventilated cage rack. *Comp Med* 54:382-392.
- Cunliffe-Beamer TL, Les EP. 1983. Effectiveness of pressurized individually ventilated (PIV) cages in reducing transmission of pneumonia virus of mice (PVM) (abstr). *Lab Anim Sci* 33:495.
- Davisson M. 2006. Federation of International Mouse Resources: Global networking of resource centers. *Mamm Genome* 17:363-364.
- Disteche CM, Eicher EM, Latt SA. 1979. Late replication in an X-autosome translocation in the mouse: Correlation with genetic inactivation and evidence for selective effects during embryogenesis. *Proc Natl Acad Sci U S A* 76:5234-5238.
- Dutko FJ, Oldstone MB. 1979. Murine cytomegalovirus infects spermatogenic cells. *Proc Natl Acad Sci U S A* 76:2988-2991.
- Endoh K, Mochida K, Ogonuki N, Ohkawa M, Shinmen A, Ito M, Kashiwazaki N, Ogura A. 2007. The developmental ability of vitrified oocytes from different mouse strains assessed by parthenogenetic activation and intracytoplasmic sperm injection. *J Reprod Dev* 53:1199-1206.
- Epifano O, Dean J. 1994. Biology and structure of the zona pellucida: A target for immunocontraception. *Reprod Fertil Dev* 6:319-330.
- Glenister PH, Whittingham DG, Wood MJ. 1990. Genome cryopreservation: A valuable contribution to mammalian genetic research. *Genet Res* 56:253-258.
- Gordon S, Fisher SW, Raymond RH. 2001. Elimination of mouse allergens in the working environment: Assessment of individually ventilated cage systems and ventilated cabinets in the containment of mouse allergens. *J Allergy Clin Immunol* 108:288-294.
- Green DP. 1997. Three-dimensional structure of the zona pellucida. *Rev Reprod* 2:147-156.
- Gunasena KT, Villines PM, Critser ES, Critser JK. 1997. Live births after autologous transplant of cryopreserved mouse ovaries. *Hum Reprod* 12:101-106.
- Gwatkin RB. 1963. Effect of viruses on early mammalian development. I. Action of Mengo encephalitis virus on mouse ova cultivated in vitro. *Proc Natl Acad Sci U S A* 50:576-581.
- Gwatkin RB. 1966. Effect of viruses on early mammalian development. III. Further studies concerning the interaction of Mengo encephalitis virus with mouse ova. *Fertil Steril* 17:411-420.
- Gwatkin RB. 1967. Passage of mengovirus through the zona pellucida of the mouse morula. *J Reprod Fertil* 13:577-578.
- Hani T, Tachibe T, Shingai S, Kamada N, Ueda O, Jishage K. 2006. Fertility of mice receiving vitrified adult mouse ovaries. *Reproduction* 131:681-687.
- Hare WCD. 1990. Design and analysis of research on infectious disease transmission by embryos. *Theriogenology* 33:51-57.
- Heggie AD, Gaddis L. 1979. Effects of viral exposure of the two-cell mouse embryo on cleavage and blastocyst formation in vitro. *Pediatr Res* 13:937-941.
- Hsu CC, Wobus CE, Steffen EK, Riley LK, Livingston RS. 2005. Development of a microsphere-based serologic multiplexed fluorescent immunoassay and a reverse transcriptase PCR assay to detect murine norovirus 1 infection in mice. *Clin Diagn Lab Immunol* 12:1145-1151.
- IATA [International Air Transport Association]. 2007. *Live Animal Regulations*, 34th ed. Montreal: IATA.
- Karst SM, Wobus CE, Lay M, Davidson J, Virgin HWT. 2003. STAT1-dependent innate immunity to a Norwalk-like virus. *Science* 299:1575-1578.
- Kiessling AA, Crowell R, Fox C. 1989. Epididymis is a principal site of retrovirus expression in the mouse. *Proc Natl Acad Sci U S A* 86:5109-5113.
- Kyuwa S. 1997. Replication of murine coronaviruses in mouse embryonic stem cell lines in vitro. *Exp Anim* 46:311-313.
- Kyuwa S, Xiao Y, Toyoda Y, Sato E. 1997. Characterization of embryonic stem-like cell lines derived from embryoid bodies. *Exp Anim* 46:11-16.
- Lavilla-Apelo C, Kida H, Kanagawa H. 1991. The effectiveness of trypsin treatment to remove Sendai virus adhering to the zona pellucida of mouse preimplantation embryos. *Jpn J Vet Res* 39:133-141.
- Lavilla-Apelo C, Kida H, Kanagawa H. 1992. The effect of experimental infection of mouse preimplantation embryos with paramyxovirus Sendai. *J Vet Med Sci* 54:335-340.
- Lee H, Purdy GA, Riley LK, Livingston RS. 2007. Efficacy of disinfectants against MVM- and MNV-contaminated surfaces. *JAALAS* 46:94-95.
- Lipman NS. 1999. Isolator rodent caging systems (state of the art): A critical view. *Contemp Top Lab Anim* 38:9-17.
- Lipman NS, Corning BF, Saifuddin M. 1993. Evaluation of isolator caging systems for protection of mice against challenge with mouse hepatitis virus. *Lab Anim* 27:134-140.
- Livingston RS, Riley LK. 2003. Diagnostic testing of mouse and rat colonies for infectious agents. *Lab Anim Europe* 3:38-47.
- Livingston R, Riley L, Bauer B, Besch-Williford C. 2004. Polymerase chain reaction (PCR) testing of biological materials for rodent pathogens as an alternative to the mouse antibody production (MAP) test. (Abstract) 9th FELASA Symposium 2004. p 38.
- Lock LF, Keshet E, Gilbert DJ, Jenkins NA, Copeland NG. 1988. Studies of the mechanism of spontaneous germline ecotropic provirus acquisition in mice. *EMBO J* 7:4169-4177.
- Mahabir E, Jacobsen K, Brielmeier M, Peters D, Needham J, Schmidt J. 2004. Mouse antibody production test: Can we do without it? *J Virol Methods* 120:239-245.
- Mahabir E, Bulian D, Needham J, Mayer A, Mateusen B, Soom AV, Nauwynck H, Schmidt J. 2007a. Transmission of mouse minute virus (MVM) but not mouse hepatitis virus (MHV) following embryo transfer with experimentally exposed in vivo-derived embryos. *Biol Reprod* 76:189-197.
- Mahabir E, Bensch S, Brielmeier M, Schmidt J. 2007b. Prevalence of mouse norovirus (MNV) in a large breeding and experimental mouse facility. (Abstract) 10th FELASA Symposium, Cernobbio, Italy 2007. p 99.
- Mahabir E, Bulian D, Schmoeller R, Needham J, Schmidt J. 2008. Production of virus-free seronegative pups from murine embryos arising from in vitro fertilization with mice minute virus-exposed spermatozoa. *Biol Reprod* 78:53-58.
- Mims CA. 1966. Immunofluorescence study of the carrier state and mechanism of vertical transmission in lymphocytic choriomeningitis virus infection in mice. *J Pathol Bacteriol* 91:395-402.
- Mukherjee AB, Cohen MM. 1970. Development of normal mice by in vitro fertilization. *Nature* 228:472-473.
- Müller B, Klemm U, Mas Marques A, Schreier E. 2007. Genetic diversity and recombination of murine noroviruses in immunocompromised mice. *Arch Virol* 152:1709-1719.
- Nevalainen T, Berge E, Gallix P, Jilge B, Melloni E, Thomann P, Waynforth B, van Zutphen LFM. 1999. FELASA guidelines for education of specialists in laboratory animal science (Category D): Report of the Federation of Laboratory Animal Science Association's Working Group on Education of Specialists (Category D) accepted by the FELASA Board of Management. *Lab Anim* 33:1-15.
- Nicklas W, Weiss J. 2000. Survey of embryonic stem cells for murine infective agents. *Comp Med* 50:410-411.
- Nicklas W, Baneux P, Boot R, Decelle T, Deeny AA, Fumanelli M, Illgen-Wilcke B. 2002. Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. *Lab Anim* 36:20-42.
- Nicklas W, Mauter P, Schneider E, Wobus CE. 2006. Mouse Norovirus (MNV): erste Untersuchungsergebnisse (Abstract) 44. GV-SOLAS Annual Meeting, Freiburg, Germany. p 55-56.
- NRC [National Research Council]. 2006. *Guidelines for the Humane Transportation of Research Animals*. Washington: National Academies Press.
- Obernier JA, Baldwin RL. 2006. Establishing an appropriate period of acclimatization following transportation of laboratory animals. *ILAR J* 47:364-369.
- Okumura A, Machii K, Azuma S, Toyoda Y, Kyuwa S. 1996. Maintenance

- of pluripotency in mouse embryonic stem cells persistently infected with murine coronavirus. *J Virol* 70:4146-4149.
- Perdue KA, Green KY, Copeland M, Barron E, Mandel M, Faucette LJ, Williams EM, Sosnovtsev SV, Elkins WR, Ward JM. 2007. Naturally occurring murine norovirus infection in a large research institution. *JAALAS* 46:39-45.
- Peters DD, Marschall S, Mahabir E, Boersma A, Heinzmann U, Schmidt J, Hrabe de Angelis M. 2006. Risk assessment of mouse hepatitis virus infection via in vitro fertilization and embryo transfer by the use of zona-intact and laser-microdissected oocytes. *Biol Reprod* 74:246-252.
- Reetz IC, Wullenweber-Schmidt M, Kraft V, Hedrich HJ. 1988. Rederivation of inbred strains of mice by means of embryo transfer. *Lab Anim Sci* 38:696-701.
- Scavizzi F, Raspa M. 2004. Tissue distribution and duration of mouse hepatitis virus in naturally infected immunocompetent ICR (CD-1) and immunodeficient athymic nude-nu mouse strains used for ovarian transplantation and in vitro fertilization. *Lab Anim* 38:189-199.
- Scavizzi F, Raspa M. 2006. *Helicobacter typhlonius* was detected in the sex organs of three mouse strains but did not transmit vertically. *Lab Anim* 40:70-79.
- Schoondermark-van de Ven EM, Philipse-Bergmann IM, van der Logt JT. 2006. Prevalence of naturally occurring viral infections, *Mycoplasma pulmonis* and *Clostridium piliforme* in laboratory rodents in Western Europe screened from 2000 to 2003. *Lab Anim* 40:137-143.
- Shaw JM, Nakagata N. 2002. Cryopreservation of transgenic mouse lines. *Methods Mol Biol* 180:207-228.
- Stringfellow DA, Seidel SM, eds. 1998. *Manual of the International Embryo Transfer Society*. Savoy: IETS.
- Suzuki H, Yorozu K, Watanabe T, Nakura M, Adachi J. 1996. Rederivation of mice by means of in vitro fertilization and embryo transfer. *Exp Anim* 45:33-38.
- Swallow J, Anderson D, Buckwell AC, Harris T, Hawkins P, Kirkwood J, Lomas M, Meacham S, Peters A, Prescott M, Owen S, Quest R, Sutcliffe R, Thompson K. 2005. *Guidance on the transport of laboratory animals*. Report of the Transport Working Group established by the Laboratory Animal Science Association (LASA). Tamworth UK: LASA.
- Takahashi E, Miyoshi I, Nagasu T. 2001. Rescue of a transgenic mouse line by transplantation of a frozen-thawed ovary obtained postmortem. *Contemp Top Lab Anim* 40:28-31.
- Tebourbi L, Testart J, Cerutti I, Moussu JP, Loeuillet A, Courtot AM. 2002. Failure to infect embryos after virus injection in mouse zygotes. *Hum Reprod* 17:760-764.
- Tuffrey M, Zisman B, Barnes RD. 1972. Sendai (parainfluenza 1) infection of mouse eggs. *Br J Exp Pathol* 53:638-640.
- Van Keuren ML, Saunders TL. 2004. Rederivation of transgenic and gene-targeted mice by embryo transfer. *Transgenic Res* 13:363-371.
- Wasserman PM, Liu C, Litscher ES. 1996. Constructing the mammalian egg zona pellucida: Some new pieces of an old puzzle. *J Cell Sci* 109 (Pt 8):2001-2004.
- Weinert D, Eimert H, Erkert HG, Schneyer U. 1994. Resynchronization of the circadian corticosterone rhythm after a light/dark shift in juvenile and adult mice. *Chronobiol Int* 11:222-231.
- Yokoyama M, Akiba H, Katsuki M, Nomura T. 1990. Production of normal young following transfer of mouse embryos obtained by in vitro fertilization using cryopreserved spermatozoa. *Jikken Dobutsu* 39:125-128.