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Expert information

From the Working Group on Hygiene

Hygienic risk in the Import of Rodents – Rederivation Strategies

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Content

1 Introduction

Today, mice and rats with a defined hygienic status are available from commercial suppliers. Additionally, a wide range of genetically modified animals are generated in scientific institutions. Such animals are used and exchanged by scientists worldwide. For a facility to establish its own colonies, animals are often received from different sometimes non-commercial animal facilities with differing hygienic statuses. These animal exchanges have to be organised carefully to eliminate the hygienic risk for recipient facilities.

2 General considerations in the import of rodents

Every animal import involves a potential risk of introducing unwanted viruses, bacteria, fungi or parasites. This includes not only pathogenic microorganisms, but also opportunistic microorganisms, which were not tested for or are not on the health certificate. These microorganisms include non-pathogenic protozoa and certain *Enterobacteriaceae*, *Staphylococcus* species, and *Streptococcus* species. Each recipient facility must define its own hygienic standards. When the sender has demonstrated that hygienic standards meet the recipient's requirements, animals and sperm/oocytes can be imported live or in a frozen state (see 3.4). The import of embryos or sperm is highly preferred over transporting live animals not only for hygienic and organisational reasons but also because of animal welfare concerns.

3 Risk assessment of microorganism carry-over

This risk is significantly influenced by:

- Facility of origin
- Health certificate validity
- Shipment conditions
- Methods of importing animals to the recipient facility

3.1 Facility of origin

Animals can come from commercial suppliers and experimental facilities. The risks of microorganism carry-over can vary between different types of facilities.

3.1.1 Import of rodents from commercial suppliers

Commercial suppliers apply their internal standards, which are generally high, to husbandry and hygienic monitoring. However, these standards might differ between breeders, hygienic units and the locations of their facilities. Despite high husbandry standards at commercial breeding facilities it is recommended that recipients thoroughly review the health status information provided and compare it with their own standards. Additionally, there are reports of sporadic outbreaks of infection in commercial breeding facilities. Therefore, animals with a health certificate may not necessarily be free of infections with unwanted microorganisms. When purchasing animals from a commercial source, foster mothers, sentinels or animals used for backcrossing may carry microorganisms like *Staphylococcus aureus*. Such microorganisms often go unreported on health certificates.

3.1.2 Import of rodents from experimental facilities

The microbiological quality of laboratory animals in experimental animal facilities has markedly increased. However, it should be noted that not every facility provides reliable information regarding the microbiological status of their animals. This can be because they either do not conduct sufficiently frequent microbiological testing or because their monitoring does not meet established standards (see FELASA recommendations[1]). The GV-SOLAS publication "Hygienic monitoring of mice and rats in various housing systems (2010)" [2] gives details about suitable health monitoring for different housing conditions. Even when frequent testing is conducted, facilities often cannot supply reliable health certificates. Thus, import examinations at recipient facilities may reveal infections with undesired microorganisms. Therefore, there is a high risk of introducing microorganisms into the recipient facilities when importing live animals from experimental facilities.

3.1.3 International Import of rodents

International collaboration is essential to biomedical research. The shipment of rodents (by air or road) is crucial, especially when hygiene is concerned, because the risk of microbial contamination increases with transportation time. The stress caused by shipment can influence the immune response of animals to potential pathogen exposure as documented by serological controls conducted directly after shipment arrival[3]. Hygienic monitoring for laboratory animals in Europe has been harmonised due to the FELASA recommendations, however there is no comparable American or Asian standard. The lack of international standards increases the risk of introducing infectious or zoonotic agents into experimental facilities (e.g. Hantavirus)[4]. When comparing hygienic monitoring results, differences could occur due to:

- different sampling and detection methods (e.g. serology, culture techniques, PCR)
- different microorganisms tested based on local prevalence

Therefore, the GV-SOLAS Working Group on Hygiene explicitly recommends the use of a standardised format in order to improve and facilitate the interpretation of health monitoring reports when importing rodents in a European and international context[5].

3.2 Health certificate validity

Before ordering animals, a detailed health certificate based on FELASA recommendations[1] should be obtained to evaluate any potential risk of infection from these animals. This certificate should contain the following information:

- number of tested animals for the period of at least 18 months prior to shipment
- frequency of tests
- range of pathogens tested
- testing methods
- all applicable testing results
- testing laboratory
- facility's husbandry conditions

GV-SOLAS has provided an example of a standardised health monitoring report format[5].

The recipient facility should provide the sender a list of all microorganisms not on the FELASA list which are not accepted at their facility. The recipient must request that the sender test for these agents.

Special attention should be paid to genetically modified animals where the modification might influence a multitude of physiological parameters. E.g. it is possible that modifications produce different effects on the immune system, including the formation of immunological defects or the suppression of the immune system. This can either increase the susceptibility to certain microorganisms or result in a lowered immunological reaction including complete lack thereof, which can lead to false-negative serological results. Hence, special care in the microbiological screening of genetically modified rodent colonies should be taken to include immunocompetent animals that can be analysed under the same conditions.

3.3 Shipment conditions

When shipping animals, the utmost care should be taken to avoid infection of animals during shipment. I.e., the cages or boxes for shipment must be equipped with filters that can prevent the intrusion of any infectious agents. Additionally, the shipment of genetically modified animals has to be conducted in closed shatterproof containers to prevent the potential escape of animals. The sender is responsible for the choice of a suitable container and packaging (see publication of the GV-SOLAS[6]). The recipient should check the containers carefully after arrival. Even in the case of small damages, the animals should be regarded as potentially contaminated.

3.4 Importing rodents into the recipient facility

Live animals can generally be directly introduced into the recipient`s facility. However, direct imports always involve a higher risk of contamination with unwanted microorganisms in comparison to rederivation. Live animal transport should only be considered under the following conditions:

- high quality health certificates
- an operational quarantine unit is available at the recipient’s facility
- the imported animals test free of unwanted microorganisms after arrival (see 3.4.1)

Furthermore, it is crucial that health certificates are evaluated by an expert. Any animal with insufficient information about its microbiological status represents a considerable risk of carry-over of unwanted microorganisms. These animals should be regarded as potentially infected and therefore, best practice recommends introduction into the animal facility only via rederivation.

Comparing the time and costs associated with rederivation of a mouse strain into a facility and those of the live import of animals, it is evident that rederivation is not only more time-consuming and expensive, but also requires specific infrastructure. The required time for introduction via quarantine is a minimum of 8 to 12 weeks, exclusive of time for hygienic testing, as opposed to 3 to 6 months for rederivation. However, an accidental carry-over of microorganisms by direct import results in higher effort and time to eradicate these unwanted microorganisms, which leads to substantially higher costs.

3.4.1 Procedure for importing live rodents

The import of live animals is only recommended if the following conditions can be met:

- the health certificate meets the recipient's standards
- the husbandry conditions guarantee that the hygienic status of the animals can be maintained until arrival
- security precautions have to be defined

Animals should be introduced into a quarantine unit, ideally one equipped with isolators or individually ventilated caging (IVC) systems. When used properly, these caging systems will protect from the spread of microorganisms and minimize the risk of transmission into other animal areas. The quarantine must be self-contained and should be in a separate location. While the negative air pressure of quarantine units can provide isolation from the neighbouring animal units, negative air pressure could increase the risk of infection within the quarantine. Only the imported animals should be housed in the quarantine unit. Due to the increased hygienic risk, designated animal care takers who have no contact with other research animals should care for the animals. No other staff should have access during the quarantine period.

The preferred method of hygienic monitoring is individual testing (for serological testing only if the mice are immunocompetent). Alternatively, imported cohort animals can be used for direct testing. The danger of transmitting microorganisms to the quarantined animals increases when using mice imported from other sources or areas as sentinels.

To indicate a potential infection of the imported animals, sentinel animals should ideally be exposed as contact sentinels. These should have a minimum exposure time of 8-12 weeks before being tested. This is the required safety period for antibody production after late infection, namely infections occurring in the exporting unit between the last scheduled testing date and the date of shipment, infection during shipment or infection in the recipient unit. For possible modes of transmission of different microorganisms see the GV-SOLAS recommendation[2]. In comparison to open-caging systems, the use of sentinels in colonies maintained in IVCs or isolators makes sentinel-based information on the microbiological status of the quarantined animals unreliable. For information on the correct use of sentinels see GV-SOLAS publication[7] and Lipman and Homberger[8]. Recent publications indicate that exhaust air dust samples can be used as an additional source of health monitoring information[9-11].

Testing is to be conducted according to internal standards (serology, bacteriology, parasitology). In addition to the testing of sentinel samples, blood, swabs and faeces collected directly from the imported animals as well as exhaust air dust samples can be used for diagnostic purposes.

After careful interpretation of results by experts, the animals can be imported into the recipient's facility, providing the results meet their standards. If this is not the case, a rederivation of the imported animals is required (see section 4).

The risk of accidentally transferring microorganisms into the facility depends on various factors:

- pathogen associated factors such as prolonged or intermittent excretion
- probability of transmission to sentinel animals
- genetic influence (e.g. strain specific resistance against particular pathogens) or the influence of age on seroconversion or excretion

- degree of sensitivity of testing method

Microorganisms with increased risk due to the above include parvoviruses[12,13], pinworms, protozoa, *Pasteurellaceae* and *Helicobacter* spp.[14]. When microorganisms are found in either sentinels or quarantined animals, the use of drugs to eliminate unwanted agents is unsuitable. Treatment against parasites is only recommended in exceptional cases (see GV-SOLAS recommendation[15]).

3.4.2 Import of gnotobiotic rodents (germ-free, germ-associated)

When importing gnotobiotic rodents the animals can be used in three ways:

- for starting a gnotobiotic colony in the recipient facility[16]
- for experiments in a gnotobiotic context
- for starting a new colony

When working in a gnotobiotic context, extended screening for infectious agents (e.g. according to FELASA recommendations) is not necessary. Hence, health monitoring of gnotobiotic animals is aimed at either demonstrating the absence of microorganisms or revealing the presence of germ-associated microorganisms. For this, regular monitoring using 16S rRNA sequencing is recommended[16]. Further testing should focus on agents that are easily introduced e.g. environmental bacteria or fungi.

For starting a new colony, gnotobiotic animals are regarded as hygienically acceptable if the microorganisms used for association correspond with the hygienic status of the facility. Health certificates with this information are prerequisites for import. However, the information provided by the sender should be verified by the recipient. In this case, the imported animals should be quarantined in an isolator with positive air pressure until completion of testing. The animals can only be introduced into the facility if the hygienic quality is sufficient.

4 Importing animals by rederivation

Rederivation can be performed by the following methods:

- Embryo transfer (with *ex vivo* collected embryos or *in vitro* produced embryos)
- Hysterectomy
- Neonatal transfer (cross-fostering)

These should be done in a designated area (isolator, physically separated room). The rederived animals should only be transferred into the breeding or husbandry area of the facility after the microbiological quality is tested. Prior to transfer foster mothers should be screened after weaning their pups. Providing they meet the facility's hygienic standards, the offspring can be imported.

4.1 Embryo transfer (with *ex vivo* collected or *in vitro* derived embryos)

The following methods of embryo transfer are considered safe techniques provided all essential hygienic precautions are taken[17-22]:

- *ex vivo*: collected live embryos or cryopreserved embryos
- *in vitro*: *in vitro* fertilization (IVF) with live or cryopreserved sperm

All live animals to be rederived must be quarantined. The quarantine procedure for imported rodents (3.4.1) is the same for embryo donors as well as for sperm donors. In brief, imported animals should be quarantined either in negative air pressure isolators or IVCs, which must be located in physically separated animal units until collection of embryos or sperm.

Different microorganisms like e.g. LCMV (lymphocytic choriomeningitis virus), MCMV (mouse cytomegalovirus), MHV (mouse hepatitis virus), MNV (murine norovirus), MPV (mouse parvovirus), MVM (minute virus of mice), Polyoma virus, and Sendai virus have been detected in ovaries, oocytes or embryos[12,17,23-31]. It can be assumed, however, that the risk of microorganism transmission by transfer of embryos with intact zona pellucida is low if sufficient washing of embryos is done (i.e. 10 washes with a minimum dilution of 1:100 between the wash steps[17,18,32]). Therefore, freshly collected or cryopreserved embryos can be introduced directly into the dedicated embryo transfer unit after respective washing steps. The use of cryopreserved embryos not only reduces the risk of introduction of microorganisms when importing live animals, but also avoids distress caused by shipment.

Additionally, embryo transfer can be performed by the use of embryos derived by *in vitro* fertilisation. For that, fresh or cryopreserved sperm can be used. Both the male reproductive organs and sperm can also transmit different microorganisms e.g. MCMV, MHV, MNV, MPV, MVM, TMEV (Theiler's murine encephalomyelitis virus), and *Helicobacter* species[12,28,29,31,33-35]. It is impossible to remove microorganisms from the sperm sample by the use of percoll gradient centrifugation[29] or by washing[7]. However, virus-free seronegative offspring can be produced through *in vitro* fertilisation with virus-contaminated sperm by following correct washing protocol[17,18,30,32]. The method of IVF with consecutive embryo transfer is very well established today[36] and has proven to be an excellent alternative to the import of live mice or the transfer of *ex vivo* collected embryos.

After washing procedure embryos can be implanted to pseudopregnant foster mothers that meet the facility's hygienic standards.

Due to a residual risk of contamination separate housing of the foster mothers is necessary. Foster mothers should be kept in a way that contamination of the rest of the facility is prevented.

4.2 Rederivation by hysterectomy

Rederivation by aseptic hysterectomy (gnotobiotechnique) should ideally be conducted in isolators with positive air pressure. It requires highly trained staff, especially when animals should be rederived to germ-free status.

To conduct a hysterectomy, the infected or sero-positive pregnant mother has to be euthanised as close to the estimated birth date as possible. In order to eliminate any microorganisms that could be present on the surface of the uterus, placenta and chorion, the donor is dipped into disinfection solution (e.g. iodophore solution) with decreasing concentrations and the dipping process is repeated. The complete uterus is removed within the dipping bath, only then can the fetuses be dissected from the uterus in a subsequent dipping bath. Either the closed uterus or the dissected fetuses enclosed in the chorion membrane are introduced into an isolator with a germ-free or specified pathogen-free foster mother that had delivered shortly before hysterectomy[37-39]. All chorionic and amniotic membranes have to be removed in this step. The

pups should be carefully cleaned with nesting material from the cage to increase acceptance by the foster mother.

Hysterectomy, although similar to a neonatal transfer, is not applicable for every agent. This method is only successful when there is no transplacental transmission of the agent to the fetus. Therefore, it is advisable to check the donor animals in advance for the vertical transmission of unwanted microorganisms. Hysterectomy is, for instance, inappropriate for animals infected with LCMV[23,40]. A diaplacental transmission of MCMV can be assumed[41]. There are also reports of transmission of parvoviruses[42,43]. Diaplacental transmission of MHV, at least experimentally, has also been described[44,45]. However, it can be assumed that this is a highly unlikely method of transmission in a laboratory[41a].

Experts do not recommend hysterectomy when immunodeficient mice carry e.g. *Pasteurella pneumotropica* or *Helicobacter hepaticus*[20,36,46]. Necessary precautions for rederivation of mice infected with parasites or bacteria are comparable to neonatal transfer (see section 4.3).

4.3 Neonatal transfer (cross-fostering)

Neonatal transfer signifies the transfer of newborn pups from an infected and/or seropositive mother to a specified pathogen-free mother. This method is not useful for the elimination of every agent and requires that no infection has occurred before, during, or shortly after the birth of the newborn offspring. Therefore, neonatal transfer should only be done within the first 24 hours after birth[47]. Furthermore, prior to transfer, pups can be dipped into a disinfection solution (e.g. iodophore solution) for a few seconds to flush away or kill any microorganisms on their bodies[48,49].

Success rates are highest with immunocompetent animals because they produce maternal antibodies and there is low probability of a diaplacental transmission. Rederivation by cross fostering is especially successful when the donor mother is infected with microorganisms excreted only for a short time (e.g. MHV, murine rotavirus) or microorganisms transmitted via the faecal-oral route (e.g. MNV, TMEV, murine rotavirus, *Helicobacter* species)[48-52]. Diets containing antibiotics should be given to the pregnant mothers, foster mothers and pups to eliminate the *Helicobacter* species[47,53,54]. A combination of neonatal transfer and medication (Ivomec®) has been reported to successfully control mites in mice[55].

In conclusion, well-established rederivation strategies are available to date. However, the hygienic risk in the import of rodents into facilities cannot be eliminated. Further research is required to ensure, that facilities hygienic standards are not threatened by the import of new mouse strains.

5 References

1. Mähler M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, Raspa M. 2014. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim 48:178–192.
2. GV-SOLAS, Ausschuss für Hygiene. 201. Hygienic monitoring of mice and rats in various housing systems. http://www.gv-solas.de/fileadmin/user_upload/pdf_publikation/Hygiene/hyg_mikrobiol-monito-hous2010.pdf
3. Aguila HN, Pakes SP, Lai WC, Lu YS. 1988. The effect of transportation stress on splenic natural killer cell activity in C57BL/6J mice. Lab Anim Sci 38:148-151.
4. Nicklas W. 2008. International harmonization on health monitoring. ILAR J 49:338-346.
5. GV-SOLAS, Ausschuss für Hygiene. 2018 Harmonisation of Health Monitoring Reports. http://www.gv-solas.de/fileadmin/user_upload/pdf_stellungnahme/2018stell_hyg-Harmonisation_e.pdf
6. GV-SOLAS, Ausschuss für Genetik und Zucht. 2016. Empfehlungen zum Transport gentechnisch veränderter Mäuse und Ratten der Risikogruppe 1. http://www.gv-solas.de/fileadmin/user_upload/pdf_publikation/Genetik/201606Transport-GV-Tiere.pdf (2016)
7. GV-SOLAS, Ausschuss für Hygiene. 2014. Zur Aussagekraft von Gesundheitszeugnissen: Kritische Anmerkungen zum Einsatz von Sentinels zur Bestimmung des Infektionsstatus in Labortierhaltungen. http://www.gv-solas.de/fileadmin/user_upload/pdf_publikation/Hygiene/201411Sentinels_Nicklas.pdf (2014).
8. Lipman NS, Homberger FP. 2003. Rodent quality assurance testing use of sentinel animal systems. Lab Anim 32:36-42.
9. Miller M, Brielmeier M. 2018. Environmental samples make soiled bedding sentinels dispensable for hygienic monitoring of IVC-reared mouse colonies. Lab Anim. 52(3):233-239.
10. Miller M, Ritter B, Zorn J, Brielmeier M. 2016. Exhaust air dust monitoring is superior to soiled bedding sentinels for the detection of Pasteurella pneumotropica in individually ventilated cage systems. J Am Assoc Lab Anim Sci 55(6):775-781.
11. Zorn J, Ritter B, Miller M, Kraus M, Northrup E, Brielmeier M. 2017. Murine norovirus detection in the exhaust air of IVCs is more sensitive than serological analysis of soiled bedding sentinels. Lab Anim. 51(3):301-310.
12. Janus LM, Smoczek A, Hedrich HJ, Bleich A. 2009. Risk assessment of minute virus of mice transmission during rederivation: detection in reproductive organs, gametes, and embryos of mice after in vivo infection. Biol Reprod 81:1010-1015.
13. Janus LM, Bleich A. 2012. Coping with parvovirus infections in mice: health surveillance and control. Lab Anim 46:14-23.
14. 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.
15. GV-SOLAS, Ausschuss für Hygiene. 2013 Prophylaktische und therapeutische Maßnahmen bei ausgewählten Infektionen von Labornagern und Kaninchen. http://www.gv-solas.de/fileadmin(user_upload/pdf_publikation/Hygiene/hyg-prophylak_0313.pdf

16. Nicklas W, Keubler L, Bleich A. 2015. Maintaining and Monitoring the Defined Microbiota Status of Gnotobiotic Rodents ILAR J 56(2):241–249.

17. Mahabir E, Bulian D, Needham J, Schmidt J. 2009. Lack of transmission of mouse minute virus (MMV) from in vitro produced embryos to recipients and pups due to the presence of cumulus cells during the in vitro fertilisation process. Biol Reprod 81:531- 538.

18. Mahabir E, Bulian D, Schmöller R, Needham J, Schmidt J. 2008. Production of virus-free seronegative pups from murine embryos arising from in vitro fertilisation with mouse minute virus-exposed spermatozoa. Biol Reprod 78:53- 58.

19. 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.

20. 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.

21. Okamoto M, Masumoto T. 1999. Production of germfree mice by embryo transfer. Exp Anim 48:59-62.

22. Van Keuren ML, Saunders TL. 2004. Rederivation of transgenic and gene-targeted mice by embryo transfer. Transgenic Res 13:363-371.

23. 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.

24. Tuffrey M, Zisman B, Barnes DR. 1972. Sendai (parainfluenza 1) infection of mouse eggs. Br J Exp Pathol 53:638-640.

25. Abramczuk J, Vorbrodt A, Solter D, Koprowski H. 1987. Infection of mouse preimplantation embryos with simian virus 40 and polyoma virus. Proc Natl Acad Sci USA 75:999-1003.

26. Lavilla-Apelo C, Kida H, Kanagawa H. 1992. The effect of experimental infection of mouse preimplantation embryos with paramyxovirus Sendai. J Vet Med 54:335-340.

27. 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.

28. 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.

29. 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.

30. Mahabir E, Bulian D, Needham J, Mayer A, Mateusen B, Van Soom A, Nauwynck H, Schmidt J. 2007. Transmission of mouse minute virus (MMV) but not mouse hepatitis virus (MHV) following embryo transfer with experimentally exposed in vivo-derived embryos. Biol Reprod 76:189-197.

31. Zhang L. 2008. Microbial pathogen contamination in mouse gametes and embryos. MSc Thesis, University of Missouri-Columbia. USA.

32. Peters DD, Marschall S, Mahabir E, Boersma A, Heinzmann U, Schmidt J, Hrabé de Angelis M. 2006. Risk assessment of mouse hepatitis virus infection via in vitro fertilisation and embryo transfer by use of zona-intact and laser-microdissected oocytes. Biol Reprod 74:246-252.

33. Dutko FJ, Oldstone MB. 1979. Murine cytomegalovirus infects spermatogenic cells. Proc Natl Acad Sci USA 76:2988-2991.

34. 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.

35. Besselsen DG, Romero-Aleshire MJ, Munger SJ, Marcus EC, Henderson KS, Wagner AM. 2008. Embryo transfer rederivation of C.B-17/Icr-Prkdc(scid) mice experimentally infected with mouse parvovirus 1. Comp Med 58:353-359.

36. 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.

37. Heinecke H. 1989. Angewandte Versuchstierkunde. Jena: VEB Gustav Fischer Verlag, pp. 66-78.

38. Van Zutphen LFM, Baumans V, Beyen AC. 1995. Grundlagen der Versuchstierkunde. Stuttgart: Gustav Fischer Verlag, pp. 131-150.

39. Hedrich HJ. 2012. The Laboratory Mouse. London: Elsevier, Academic Press, pp. 639-670.

40. National Research Council (US) Committee on Infectious Diseases of Mice and Rats. 1991. Infectious diseases of mice and rats. Washington (DC): National Academies Press (US), pp. 199-205.

41. Percy DH, Barthold SW. 2007. Pathology of laboratory rodents and rabbits. Ames (IA): Blackwell Publishing, pp.19-20.

41a. Percy DH, Barthold SW. 2007. Pathology of laboratory rodents and rabbits. Ames (IA): Blackwell Publishing, pp. 28-31.

42. Kilham L, Ferm VH. 1964. Rat virus (RV) infections of pregnant, fetal and newborn rats. Proc Soc Exp Biol Med 117:874-879.

43. Kilham L, Margolis G. 1966. Spontaneous hepatitis and cerebellar "hypoplasia" in suckling rats due to congenital infections with rat virus. Am J Pathol 49:457-475.

44. Katami K, Taguchi F, Nakayama M, Goto N, Fujiwara K. 1978. Vertical transmission of mouse hepatitis virus infection in mice. Jpn J Exp Med 48:481-490.

45. Carthew P, Wood MJ, Kirby C. 1985. Pathogenicity of mouse hepatitis virus for preimplantation mouse embryos. J Reprod Fertil 73:207-213.

46. Flynn RJ, Brennan PC, Fritz TE. 1965. Pathogen status of commercially produced laboratory mice. Lab Anim Care 69:253-448.

47. Singletary KB, Kloster CA, Baker DG. 2003. Optimal age at fostering for derivation of *Helicobacter hepaticus*-free mice. Comp Med 53:259- 264.

48. Watson J, Thompson KN, Feldman SH. 2005. Successful rederivation of contaminated immunocompetent mice using neonatal transfer with iodine immersion. Comp Med 55:465-469.

49. Compton SR. 2008. Prevention of murine norovirus infection in neonatal mice by fostering. J Am Assoc Lab Anim Sci 47:25-30.

50. Lipman NS, Newcomer CE, Fox JG. 1987. Rederivation of MHV and MEV antibody positive mice by cross-fostering and use of the microisolator caging system. Lab Anim Sci 37:195-99.

51. Truett GE, Walker JA, Baker DG. 2000. Eradication of infection with *Helicobacter* spp. by use of neonatal transfer. Comp Med 50:444-451.

52. Artwohl JE, Purcell JE, Fortman JD. 2008. The use of cross-foster rederivation to eliminate murine norovirus, *Helicobacter* spp., and murine hepatitis virus from a mouse colony. J Am Assoc Lab Anim Sci 47:19-24.

53. Jury J, Gee LC, Delaney KH, Perdue MH, Bonner RA. 2005. Eradication of *Helicobacter* spp. from a rat breeding colony. Contemp Top Lab Anim Sci 44:8-11.

54. Kerton A, Warden P. 2006. Review of successful treatment for *Helicobacter* species in laboratory mice. Lab Anim 40:115-122.

55. Huerkamp MJ, Zitzow LA, Webb S, Pullium JK. 2005. Cross-fostering in combination with ivermectin therapy: a method to eradicate murine fur mites. Contemp Top Lab Anim Sci 44:12-16.

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