Methods for improvement of laboratory animal health

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Introduction

If a pathogenic agent has been accidentally introduced into a colony, there are several options for trying to eliminate it or control the consequences of this event (Figure 1) (*Wickert et al.* 1958, *Moore & Aldred* 1978). When considering the possible methods for improving the health of laboratory animals, the routes by which transmissions of infection can occur must be identified.

The transmission of vertically transmitted agents is via the gametes. Horizontal transmission can take place between related or unrelated individuals (*Neighbour* 1977).

Transmission between the pregnant mother and the fetus is a borderline case, which can be difficult to classify.

Figure 2 gives a schematic representation of the possible routes of transmission of pathogenic organisms from the mother to the embryo/fetus/ offspring and between individuals. It should be noted that vertical transmission is often used incorrectly as a synonym for transplacental or intra uterine transmission.

Methods for improving laboratory animal health

Hysterectomy

Hysterectomy is the removal of the uterus including those fetuses, which may be in the uterus. The terms most commonly used are hysterectomy, caesarean section, caesarean derivation or rederivation. The practical and technical approaches to the use of hysterectomy has been described (*ILAR* 1970, *Bleby* 1972, *Trexler* 1982, *Gulden* 1975, *Zillmann* 1976). However, one of the most critical procedures in the hysterectomy is the estimation of the stage of pregnancy. The success of a hysterectomy is dependent on the pregnancy being as close to full term as possible. This is particularly true in animal species with a short gestation period where the fetus may gain 20 percent of its weight in the final 24 hours before parturition. Timed mating may help to estimate the time of delivery. However, the gestation period may be dependent on the litter size. The ability of the personnel to evaluate the stage of gestation by palpation and to carry out the hysterectomy procedure is therefore crucial (*Taylor et al.* 1986, *Spörri* 1987, *Zillmann* 1976). The outcome of the hysterectomy procedure will also depend on the strain subjected to hysterectomy and what strain (or species, if different) is selected as the foster mother. In most laboratories only a small percentage of the hysterectomies result in non-viable young. However, there seems to be a considerable loss of young after transfer to the foster mother. In general, the percentage of litters weaned of the total number of litters recovered varies from 0 to 80 % in mice and from 0 to 50 % in rats (*Zillmann* 1976, *Bomholtgård* 1992, *Hämmerli & Hürni* 1969).

There is evidence to show that the placenta acts as a very efficient filter and protects the fetus against many bacterial and viral agents carried by the mother. The fetus in the uterus is almost always sterile.

Therefore, the obvious way to obtain 'clean' animals is to take the young by hysterectomy under aseptic conditions and to rear the young under aseptic conditions. The original animals have to be hand-reared, but once these animals have reached maturity they can be bred and will continue to be germfree if kept in isolators. These germfree animals can be used as foster mothers for young obtained by hysterectomy so that other strains of the same, or compatible species can become germfree.

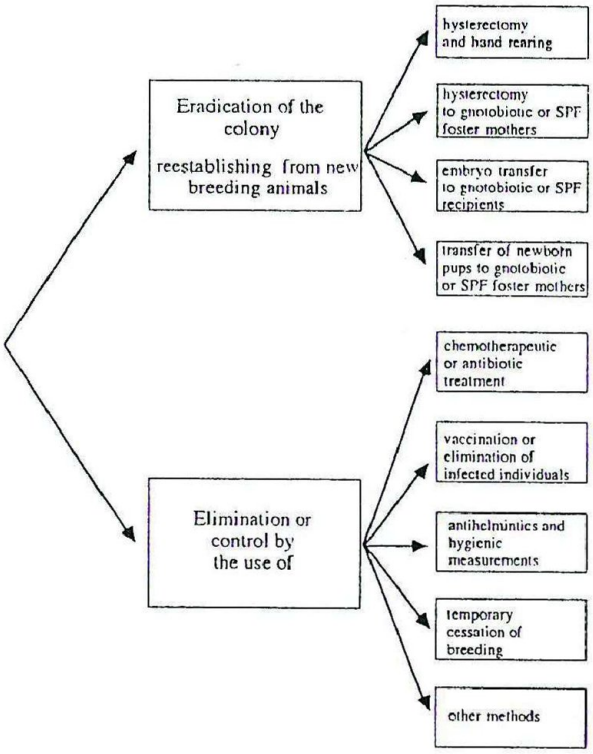

Figure 1. Summary of methods for improving laboratory animal health.

Common pathogens and parasites can be eliminated from mouse and rat colonies by use of hysterectomy.

Katami et al. (1978) found that if pregnant female mice were inoculated i.v. with Mouse Hepatitis Virus (MHV), the virus was able to cross the placenta and infect the fetus. It is, therefore most, probable that transmission of virus to the fetus only takes place in animals without antibodies and which are in the middle of pregnancy. The infection will most often result in death of the fetus (*Fujiwara et al.* 1976).

Tucker & Stewart (1976) were able to isolate Sendai virus from fetuses or newborn young after intravenous noculation of pregnant mice. However as viremia is rare in Sendai infections, transplacental transmission is unlikely.

Transmission of Lactatedehydrogenase Virus LDV may occur across the placenta. There were, however, no effects on fetuses or newborn young (*Crispens* 1965, *Notkins* 1965). The transplacental transmission of LDV is regulated by antibodies against LDV and adoptive transfer of interferon can protect fetuses from transplacental transmission (*Cafruny et al.* 1991).

Inoculation of polyoma virus into pregnant mice increase fetal mortality. However, transplacental transmission is considered to be of little or no importance in natural infections (*McCance & Mims* 1977, 1979).

In contrast to other viruses, intrauterine transmission is the most common route of transmission of Lymphocytic Choriomeningitis Virus (LCM). If a latent virus carrier state is establed in a mouse (the mouse is then immunoincompetent), transplacental transmission is very effective (*Mims* 1969, 1981, *Lehmann-Grube* 1982).

Experimental inoculation of Minute Virus of Mice (MVM) into pregnant mice resulted in the infection of the fetus (*Kilham & Margolis* 1971). Despite this, transplacental transfer of MVM is unlikely (*Parker et al.* 1980). Transplacental transmission of the other two rodent parvoviruses (Kilham Rat

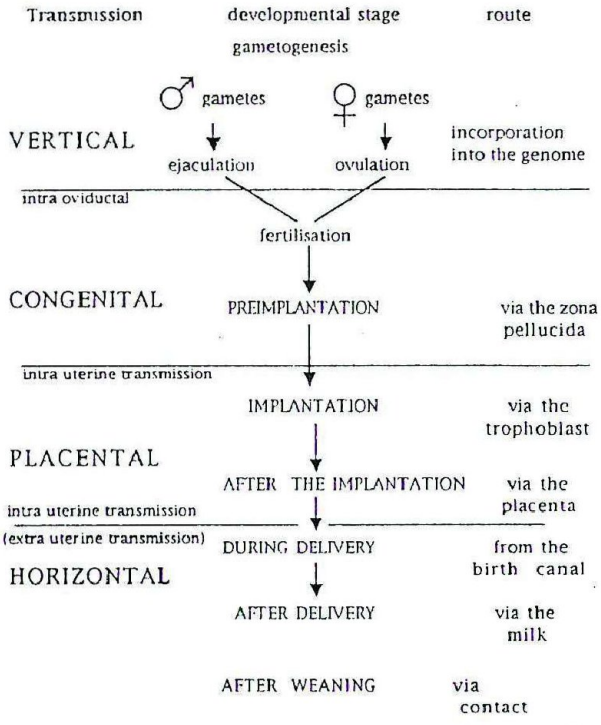

Figure 2. Schematic representation of transmission routes of infectious agents during and after pregnancy.

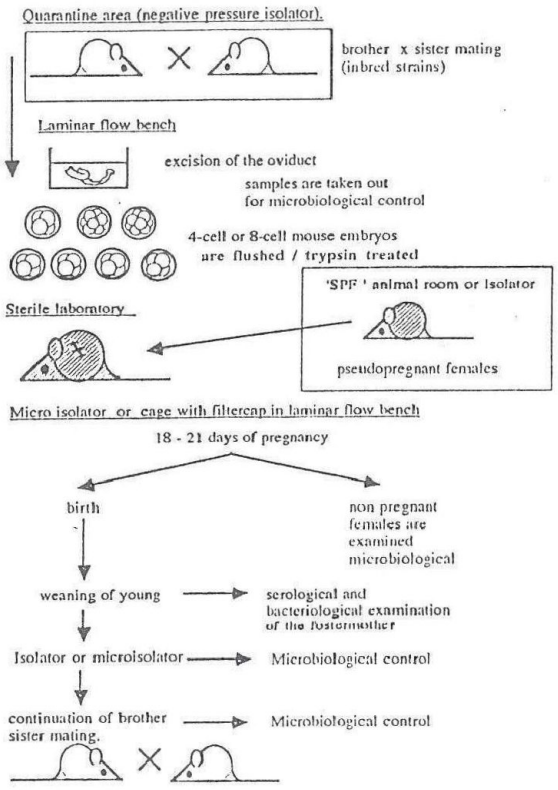

Figure 3. Schematic representation of the embryo transfer procedure for rederivation of an inbred strain of mouse.

Virus, Toolan H-1 virus) is considered to be unimportant (*Jacoby et al.* 1987, 1988, *Paturzo et al.* 1987). Ectromelia virus has also been shown to be transmitted transplacentally (*Mims* 1969, *Schwancer et al.* 1975). Infected fetuses are not viable and unlikely to develop to term.

Some pathogenic bacteria and mycoplasmas are able to inhabit the mucous membranes of animals as commensals in shorter or longer periods (latent infection), but they may also occassionally be found in those inner organs (such as the uterus) which are associated with the external mucous membranes. This seems to be the case for *Pasteurella pneumotropica* and *Mycoplasma* spp. (*Flynn et al.* 1965, 1968, *Lindsey et al.* 1982). *Fries* (1978) reported that if *Bacillus piliformis* was inoculated in pregnant female mice, the organism could be detected in the uterus, fetal membranes as well as in the fetus. Transplacental transmission was possible in the second and third week of pregnancy. It was not established whether infected fetuses could survive and develop normally. The presence of antibodies in the hysterectomy derived SPF colony and the results obtained by experimental inoculation, however, indicated that transplacental transmission could not be excluded.

Rodent colonies permanently having antibodies against *B. piliformis* may be reestablished by hysterectomy of tetracycline treated dams, to avoid transplacental transmission.

During the hysterectomy procedure there are several possible sources of contamination. The type of contaminating organism may be related to the source of contamination. A contamination caused by a leak in the surgical gloves will typically be reflected by the presence of microorganisms inhabiting the skin (*Staphylococcus* spp., *Micrococcus* spp.). *Micrococcus* spp., together with *Bacillus* spp., may be observed in airborne contamination. The presence of species of enterobacteriaceae may indicate that the intestine might have been perforated during the hysterectomy procedure. Contamination, in contrast to transplacental infections, will often be by a mixed flora.

The more serious contaminations are those involving potential or obligate pathogenic organisms. The source of contamination will be microorganisms present in the uterus (transmitted from the vagina), the vagina or the intestine. *Mycoplasma pulmonis* and *Pasteurella pneumotropica* have been found in the uterus (*Juhr et al.* 1970, *Flynn et al.* 1968), but *Flynn et al.* (1968) have also isolated *Mycoplasma pulmonis* in the fetal membrane of pregnant mice.

If the hysterectomy is performed close to natural parturition, the birth canal have opened, resulting in a possible contamination via the vagina. *Reyniers* (cit. from *Pilgrim & Parks* 1968) waited to do the hysterectomy until the first pup was born and ignored the possibility of contamination via the opening of the birth canal. The importance of the

presence of microorganisms in the uterus during hysterectomy and contamination via the vagina has not been fully evaluated.
After the hysterectomy procedure, samples may be taken from the fetal membranes and examined for the presence of infection or contamination. The isolators housing the foster mothers are screened on a regular basis for contamination or infection.

Embryo transfer

Embryo transfer (ET) involves the removal of preimplantation embryos from the donor and transfer of the embryo to a recipient at the same stage of pregnancy (or pseudo pregnancy) as the donor. Embryos are recovered from the donor after natural mating or superovulation and mating. By superovulation a greater number of eggs might be ovulated. After mating, the embryos are isolated from the oviduct or uterus, depending on the time elapsed since ovulation and fertilisation. The embryos are transferred to a pregnant or pseudopregnant female which is synchronous with the donor. During the first period after fertilisation the embryo is surrounded by a thick transparent layer, the so-called zona pellucida. The zona pellucida is impermeable to most viruses, mycoplasmas and bacteria. Just before implantation, the zona pellucida breaks, and viruses, mycoplasmas and bacteria within the lumen of the uterus may gain direct assess to the embryonic cells. Therefore, if embryos with an intact zona pellucida are removed under aseptic conditions, flushed several times in sterile medium and transferred to a pseudopregnant recipient which is SPF or germfree, transmission of many pathogens from the donor to recipient can be avoided. In order to preclude the possibility of concurrent transfer of pathogens, a combined washing and trypsin treatment is recommended (*Manual of the IETS* 1986).
In recent years the use of ET for disease control has attracted increasing interest and the possible routes of transmission of viral or other pathogens to the embryo in ET have been investigated.
Viruses may reach the female reproductive tract through the blood or with the sperm during fertilisation, as has been seen with LCM and some leukoviruses. Zona pellucida which surrounds the embryo in the first days of gestation, is considered to protect the embryo against viruses and bacteria. A number of experiments have shown that the embryos are neither influenced nor infected when they are exposed to virus *in vitro* (*Neighbour* 1977, *Biczysko et al.* 1973, *Mohanty & Bachmann* 1974, *Gwatkin* 1967, *Baskar & Eng-Shuang* 1981).
Carthew et al. (1985) examined the influence of Mouse Hepatitis Virus (MHV) on preimplantation embryos and the possibility of transmitting MHV with the embryos during ET. By transfer of the embryos, which had been flushed, infection was not transmitted. However, transfer of infected medium with embryos resulted in the formation of antibodies against MHV in the recipients. MHV could not infect embryos with intact zona pellucida.
Elimination of Sendai Virus by use of ET have also been described by *Carthew et al.* (1983).
In laboratory animal science, ET has been used for sanitising inbred strains to produce germfree mice (*Spörri* 1987) and to avoid the transfer of viruses, mycoplasmas and bacteria from the mother to the young (*Carthew et al.* 1983, 1985, *Glenister & West* 1986, *Dagnæs et al.* 1988, *Kornblat et al.* 1984, *Reetz et al.* 1987). The application of the embryo transfer technique for obtaining germfree or SPF rats has also been described (*Juhr* 1976, *Rouleau et al.* 1992).

Hygienic measures, vaccination and treatment

Elimination of infectious agents by temporary cessation of breeding.
In the case of an infection with a contagious agent spreading through the population very quickly (epizootic), the animals develop antibodies. If there is no latent carrier state, the infection is self limiting provided that no susceptible animals are introduced to the

colony during the acute phase of the disease. Examples of such infections are Sialodacryoadenitis Virus (SDAV) in rats (*Jacoby et al.* 1979). Virus is shed for only 7 days after infection and there is no latent carrier state. The infection may be eliminated by cessation of breeding or destroying all newborn pups for 6–8 weeks. In research colonies (non-breeding colonies) the same result can be obtained by a 6–8 weeks period of quarantine, during which no new animals are introduced. The animals once infected will, however, still have antibodies against SDAV.

In the case of mouse coronavirus, *Weir et al.* (1987) reported a successful elimination of MHV from a mouse colony by cessation of breeding. In contrast, however, *Nüssel* (1989) was unable to create new colonies of 6 different mouse strains, free of MHV, by discontinuation of the breeding. The discrepancy between the two reports may be due to differences in the response to MHV among mouse strains or differences in the virulence of the strains of virus. However, reinfection could not be excluded as the cause of failure in the later report. Recently, *Søndergård et al.* (1992) have described the establishment of a colony free of MHV by discontinuation of breeding by isolation of the males for 8 weeks.

Sendai virus infection may be eliminated by placing the animals under strict quarantine, removing all the young and suspending breeding for 2 months until the virus has been eliminated (*Iwai et al.* 1977). After this period, breeding may be resumed.

Isolation and selection of breeding animals.

In the case of the introduction of an infection which is less contagious and appears constantly with a lower or higher frequency throughout the population (enzootic), other methods may be applied for improving laboratory animal health. Several factors, however, have to be considered in trying to eliminate the agent, such as the development of protective antibodies, the length of time the agent is shed from the host, and the existence of a latent carrier state.

PVM is typically enzootic and since the virus carrier state and active infection lasts for only 9 days, it may be eliminated by isolating a few individual breeding pairs and selecting only seronegative animals for breeding. Alternatively, sero-positive breeding pairs which produce sero-negative young may also be isolated in microisolators to produce breeding animals free of PVM (*Horsfall & Ginsberg* 1951, *Smith et al.* 1984). TMEV infection has also been eliminated from valuable mouse stocks by foster nursing infant mice on TMEV free mice or rats (*von Magnus & von Magnus* 1948, *Lipmann et al.* 1987).

Control of rat parvovirus (Kilham rat virus, H-1 virus) might also be accomplished by selecting only seronegative breeding pairs for further breeding within the colony. However, since these viruses are very resistant and stable to dessication and disinfection this may not be effective in eliminating the agent from the colony. Complete elimination may be possible by isolating individual breeding pairs in microfilter cages and selecting sero-negative young for further breeding (*Jacoby et al.* 1979).

LDV may be eliminated from known contaminated stocks by selection of animals with normal plasma LDV concentrations and eliminating animals with elevated plasma LDV concentrations, since LDV is only shed from infected animals for a short period after infection and is not likely to be transmitted horizontally in mouse breeding colonies (*Notkins* 1965).

Encephalitozoon cuniculi may be eliminated by serologic testing of adult animals and selection of *E. cuniculi* free breeding stocks in rabbits (*Bywater & Kellett* 1978).

Vaccination.

Vaccination, though widely applied in human and veterinary medicine, has not been used to any considerable extent in laboratory rodents. Vaccination with capsid anti-

gen from simian rota virus SA-11 has been found to protect mice from diarrheal disease when challenged with mouse rotavirus (*Sheridan et al.* 1984). Vaccination against Sendai virus (*Eaton et al.* 1982, *Nedrud et al.* 1987, *Parker* 1980) and *Mycoplasma pulmonis* (*Cassell & Davis* 1978, *Taylor et al.* 1977) has also been applied. Vaccination against Ectromelia virus will protect against clinical diseases. However, it does not prevent infection or virus transmission (*Bhatt et al.* 1986). Vaccination is laborious and expensive. Furthermore vaccinated animals will still be sero-positive, in which case it is impossible to determine whether the animals have experienced a natural course of infection or have been immunised, unless the kinetics of the immune response to the vaccine has been studied in detail. The vaccination programme might also affect the immune system of the animals rendering the animals useless in immunological research.
Vaccination of breeding animals may, however, prove an efficient means of controlling and eliminating infection in breeding colonies.

Antimicrobial treatment.
In case of infections caused by bacteria *Mycoplasma pulmonis* or protozoa administration of antimicrobial drugs may help to control clinical signs, but such drugs are not curative and will not eliminate the infectious agents. Furthermore, the use of drugs may introduce variables if animals on experiment are treated. If individual animals or breeding animals are isolated and subjected to antimicrobial treatment in microisolators, elimination of some of the less resistant infectious agents might be possible. Agents resistant to desiccation and disinfection, such as *B. piliformis,* might still be difficult to eleminate by this procedure.
Succesful elimination of endoparasites (pinworm) by use of filter-top cages and repetitive treatment with antihelmintics has been reported (*Iwarsson et al.* 1988, *Wagner* 1970, *Flynn* 1973, *Hsu* 1979, *Wescott* 1982). Antihelmintics may eliminate adult worms; some may also eliminate immature worms, but no treatment is able to clear eggs indicating that hygienic measures are essential in such procedures.

Discussion and conclusion
Risk assessment and evaluation of cost benefit
Transplacental transmission of potential or obligate pathogens must be considered to be relatively rare. However, contamination during hysterectomy from adjacent mucous membranes is more likely. By the use of antibiotic treatment before hysterectomy, infectious agents are suppressed. The use of embryos provides greater security against viral and bacterial transmission, although mycoplasma might be transmitted through the embryo transfer procedure (*Iwarsson et al.* 1987). By means of antibiotics and antimicrobial drugs added to the flushing medium, the use of normal aseptic procedures and repeated washings and trypsin treatment the probability of transmission is low. The ET method eliminates uncertainty with regard to the presence of bacteria or mycoplasmas in the uterus. ET comprises two surgical procedures involving germfree or SPF animals (vasectomy of the males and embryo transfer). In each procedure there is a risk of contamination. However, in a sterile environment the probability of contamination is minimal.
A direct introduction of new breeding animals into an existing animal facility always presents a risk of concurrent introduction of pathogens. Some pathogenic organisms are subclinical and have a very low prevalence. Thus, the detection of such agents requires the examination of a relatively high percentage of the animal population (*Karasek* 1970, *Fujiwara* 1971, *Fujiwara et al.* 1976, *Schwanzer & Maess* 1983, *Allen & Nomura* 1986).
In order to detect subclinical infections, new breeding animals are maintained in a quarantine unit until it is certain that the ani-

mals are disease free or until hysterectomy can be performed. The disadvantage of such a procedure is that the animals are introduced into the domain, and may be infected with pathogens which not have been found in the colony before.

Hysterectomy may be performed in the colony of origin and the pups transported to the new colony. However, the recovered pups have to be transferred to the foster mother within a short time, and cannot survive transport over appreciable distances. Hysterectomy requires precise synchronisation of donor and recipient with respect to pregnancy and the exact timing of the hysterectomy is crucial.

By embryo transfer the ovulation and mating of the donors may be precisely synchronised with induction of pregnancy or pseudopregnancy in the recipient (*Whitten & Champlin* 1978). Moreover embryos can be maintained at room temperature for several hours, at 5°C for up to 2 days, and frozen for an indefinite period (*Glenister & West* 1986, *Scheffen et al.* 1986). Embryo transfer may prove a valuable method for introducing new breeding animals into a colony without the risk of concurrent transfer of infectious diseases. ET may also be considered in cases where the animals are infected with agents suspected of being transplantally transmitted.

The disadvantage of ET lie in the technical and practical difficulties, in establishing the technique, and that it may be difficult to obtain fertilised embryos in some inbred strains (*Yokoyama et al.* 1981). The outcome of hysterectomy and ET are difficult to compare, but seem to be approximately similar. However, ET is often considered to be the more laborious and complicated procedure.

Elimination of infectious agents by cessation of breeding may represent a valuable alternative to the radical options, since the colony need not be eliminated. However, there is some question as to the possibility of later reinfection from latently infected individuals, particularly in the case of MHV infections.

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