# Possible routes of contamination of laboratory rodents kept in research facilities

by *Werner Nicklas*
Central Animal Laboratories, German Cancer Research Centre, Heidelberg, FRG.

*Introduction*

It is generally accepted that intercurrent infections in laboratory animals may interfere with various types of research and may thus be of importance for the quality of animal experiments. There are many reports in the literature which clearly demonstrate that a number of infectious agents can be responsible for serious research complications. For that reason effective measures should be taken to standardize laboratory animals microbiologically as far as possible. For most purposes it is sufficient to use so-called 'SPF' animals. This term means that they are health-monitored and free of certain specified microorganisms, which have the potential to influence the health of animals (and humans) or the results of animal experiments. These are primarily species-specific microorganisms. However, bacteria of human origin or even environmental microorganisms may be of importance, too, especially in immunosuppressed individuals.

The design of modern laboratory animal buildings is based mainly on microbiological concepts aiming at the prevention of infections. These measures are responsible for a high percentage of expenses arising from planning and constructing an animal house, and for the equipment. Furthermore, high running costs are taken into account for energy, hygienic precautions, and personnel to avoid infections during operation.

In this paper I will focus on mice and rats. They are the most commonly used laboratory animal species and many strains of rats and mice are available in a good microbiological quality from various breeders. However, different murine pathogens are still widespread in rodent colonies throughout the world (National Research Council 1991). Compared to breeding colonies, intercurrent infections are more prevalent in experimental colonies of rodents. This fact demonstrates that keeping rodents in research facilities free of pathogens is a much more complex problem than in breeding facilities, due to various factors resulting in a higher risk of introducing pathogens or other unwanted microorganisms into experimental colonies.

In this paper some important factors will be discussed which bear the risk of introducing murine pathogens into research colonies of laboratory rodents. In contrast to commercial breeding units, animals and various experimental materials (biological materials, chemicals, etc.) need to be introduced into experimental facilities. In addition, more personnel must have access to animals due to the needs of experiments. Attempts will be made to evaluate the most important factors. A realistic control of infectious agents can only be achieved when a simultaneous attack is made on all possible routes of contamination.

*General considerations*

An appropriate *management system* for an animal facility is an important factor in prevention of contaminations as well as in their detection and control. The importance of an adequate management system is frequently underestimated compared to constructive measures. However, only the combination of both can successfully prevent the introduction of unwanted microorganisms. In addition, the types of risk factors and their importance depend on characteristics of the animal experiment. It is a major task for the management of an animal facility to understand how microorganisms might be intro-

duced or spread under the specific conditions given.

Management of all animal facilities in an institution is best centralized. This warrants that all information dealing with purchase of animals, use of experimental materials and equipment as well as performance of animal experiments is flowing through one office. This reduces the opportunity for failures of communication. A centralized management can best establish comprehensive *monitoring programmes* to evaluate important risk factors like animals and biological materials before they are introduced into a facility. The person responsible for an animal facility should know which infectious agents, if any, are coming into the facility with newly arrived animals or materials. Similarly, the management needs to know which pathogens are present in a room or a colony in order to effectively prevent transmission of microorganisms. The costs of a diagnostic laboratory should be considered a necessary part of the research programme of a sufficiently big institution performing animal experiments. Although seemingly expensive, such programmes may cost only a few percent of the whole expenses caused by an animal facility (*Martin et al.* 1990). Details about what should be considered for a comprehensive management system have been reviewed by *Lang* (1983) and *Small* (1986).

Contamination of animals used in experiments can happen in two ways. One has to distinguish between the introduction of microorganisms coming from outside into an animal house and transmission of microorganisms within a colony. Both can be influenced by the management and the housing system.

*Conventional housing systems* frequently do not take constructive or organisatory measures to efficiently reduce the risk of introduction of pathogens. Usually, the animals are not or not sufficiently monitored microbiologically, and comprehensive information on the health status may not exist. Once having been introduced, pathogens may spread within the whole facility. The likelihood of transmission is higher when animals, personnel and materials have free access to different units.

During the last decade the concept of housing animals 'behind barriers' has become more common. This term is used here to describe any physical arrangements or procedures which are set up to minimize the risk of introduction of unwanted microorganisms into a barrier unit. The *barrier concept* may be applied to a whole facility consisting of several animal rooms, corridors, autoclave, etc., or to groups of cages (for example in an isolator), or to a single cage (filter tops). Animals housed 'behind barriers' can more easily be maintained free of infectious agents than colonies maintained under conventional conditions. However, a comprehensive barrier programme can only prevent the introduction of pathogens from outside. Once a pathogen has entered a barrier unit, spreading within the unit can hardly be avoided. For that reason, a barrier programme must primarily guarantee that animals, persons and materials which have to cross barriers are free of unwanted contamination. This can best be achieved when monitoring practices are part of the barrier concept. The quality of bought-in animals must be redefined upon arrival. Frequently information on the microbiological status of animals and additional important risk factors like, for example, biological materials coming from uncharacterized sources, is needed. In addition, animal colonies must be monitored regularly to assure the absence of unwanted microorganisms. Detection or exclusion of an infection can only be achieved when appropriate diagnostic methods are used, as most intercurrent infections in rodents are subclinical. For that reason it is essential that infections are prevented, not only clinical disease.

*Risk factors*

1. Animals

The greatest risk of contamination to any animal arises from another animal of the same species. Most experimental colonies are multipurpose and must therefore house a variety of strains coming from various breeding units. If specific strains needed are not available from commercial breeders, they may be purchased from research institutions with no health monitoring programme. It has to be expected that the increasing exhange and use of specific strains or transgenic animals will be a serious challenge for experimental animal colonies in future due to the increasing need to house such animals from many sources and of variable pathogen status within one facility.

The *microbiological quality* of rodents has increased during the last decade. Meanwhile most of the breeders have screening programmes and supply their test results indicating that many commercial breeding colonies are free of murine pathogens. On the other hand, a high percentage of rodent colonies is infected with various murine viruses (*Kraft & Meyer* 1990, *Lussier & Descoteaux* 1986). *Lindsey* (1986) and others (see overview in National Research Council 1991) report a high revalence of murine viruses, parasites and bacterial pathogens even in 'barrier' facilities. For that reason animals coming from sources of unknown microbiological status should be regarded as being infected unless their status has been defined.

Animals must be protected from *exposure to pathogenic microorganisms* in the breeding unit and during shipment. Exposure to pathogens may occur in the breeder's facility if there are infected animals in some areas. Frequently, all transportation boxes are brought to one loading area for dispatch. If transported by a public carrier, they may be contaminated by mixing boxes from several sources or by contact to wild rodents. Finally, exposure to pathogens is possible at the research institution if they are improperly handled. The risk of contamination during shipment is minimized by the use of filter-protected transportation boxes. As a proper disinfection of shipping containers is usually not possible, the animals should be unpacked before being transferred into the holding area. *Martin et al.* (1990) describe that in their institution one person opens the box containing only the outside, another person reaches in and picks up the animals. We have developed containers for transportation which can be connected with a chute similar to the method described by *Eaton* (1986). This procedure relies on pressure differentials which are surmounted by animals, but not by dust and small particles.

Several organizations recommend that animals should be placed in *quarantine* upon arrival (*National Research Council 1985,* 1991; *GV-SOLAS* 1988). In practice, a consequent quarantine of all bought-in animals is often not possible. In most cases various types of experiments of various duration are performed in one barrier unit. Sometimes animals of a specific age or weight are needed which would be of no value after a quarantine period. For that reason it can be necessary to move animals to a barrier unit on a regular basis before results of thorough monitoring are available. The risk of introducing pathogens is acceptable when direct transfer is restricted to animals coming from sources of well known microbiological status. It can be minimized by housing newly arrived animals in filter cabinets or filter-top cages within the barrier area until they are proven to be pathogen-free. One should be aware that commercial breeders may have several areas supplying the same strain or stock which may be of different quality. For several reasons it is advisable not to rely exclusively on health reports supplied by the breeder. We therefore test animals sampled at random from each shipment to insure ourselves of the microbiological status of the animals.

In contrast to animals coming from well characterized sources, animals from *sources of unknown microbiological status* must be

quarantined. They are considered contaminated and must be isolated from other animals. Clinical signs cannot be expected, therefore quarantine is reasonable only when animals are thoroughly monitored for pathogens while they are in isolation. Repeated monitoring is necessary to ensure absence of pathogens because monitoring animals only on arrival does not detect an infection in the incubative phase (*Small* 1986).

Taken together, animals must be considered the most important risk factor. The risk of introducing unwanted microorganisms is acceptable when the number of suppliers is limited to as few possible and when only those breeders are chosen whose practices are in accordance with requirements as defined by the research institution. Reliable monitoring must be maintained at regular intervals to redefine the microbiological status after receipt of the animals in the user's facility. Animals coming from unknown sources must be separated and housed in strict isolation, best in isolators with negative pressure. As a rule, compromises should never be made with animals of unknown microbiological status.

## 2. Biological materials

Various reports exist in the literature of influences on animal experiments and health attributable to contaminated biological materials. Many murine viruses like minute virus of mice (MVM), K virus, mouse encephalomyelitis virus, and mouse adenovirus were first isolated from contaminated virus pools or, like polyoma virus, Kilham rat virus (KRV), and Toolan's H-1 virus, from contaminated tumours. Such contaminants can be stored frozen without any loss of infectivity and can be hazardous for humans or for laboratory animals even after decades. *Shope* (1986) reports on various complications which were caused by viruses contaminating biological materials. The problem of contamination in biological materials has gained importance due to the diagnostic or therapeutic use of monoclonal antibodies in humans (*Carthew* 1986). Contamination of murine monoclonal antibodies is an important hazard, and for that reason different authorities recommend that monoclonal antibodies intended for use in humans should be tested for murine viral contamination (*FDA* 1983, *Commission of the European Communities* 1988).

The problem of viral contamination in biological materials originating from rodents became obvious by studies from *Collins & Parker* (1972). They monitored 475 murine leukemias and tumours and found viral contamination in 69 % of the samples; 23 % were contaminated with two or more viruses. Among various contaminants, lymphocytic choriomeningitis virus (LCMV) and hantaviruses have repeatedly been found (*Bhatt et al.* 1986, *Yamanishi et al.* 1983), and outbreaks in humans associated with infected colonies of laboratory rodents or with contaminated tumours have been reported (*Bowen et al.* 1975, *Kawamata et al.* 1987).

We tested different biological materials for murine viral contamination (*Nicklas et al.* 1993). From 297 tumours examined, 25 % were contaminated. Considerable differences in the contamination rate became evident between tumours from in vitro and in vivo passages. 36.6 % of 186 tumours which had been propagated in animals were positive whereas only 6.3 % of 111 tumours propagated in vitro were contaminated. The highest rate of contamination was detected in mouse tumours. 46 % of 135 specimens of mouse origin were contaminated, and even 70.4 % of 81 samples propagated in mice were positive for murine viruses. Murine viruses were, too, found in human tumours which had been passaged in nude mice. Further, 3.7 % of 109 cell lines and 2 of 60 monoclonal antibody preparations or hybridoma cells were contaminated.

Various murine viruses like MVM, mouse hepatitis virus (MHV), KRV or reovirus 3 have been detected in biological materials.

The most frequent contaminant is lactic dehydrogenase virus (LDV) (*Collins & Parker* 1972, *Nicklas et al.* 1993). This virus causes a lifelong lasting viremia in mice without clinical signs, and each sample taken from an infected animal is contaminated with this virus. We found LDV, too, in a monoclonal antibody preparation where it caused time consuming research complications (*Nicklas et al.* 1988).
Contamination of biological materials is not restricted to viruses. Our study revealed Mycoplasma pulmonis in a cell line. Other bacteria like Pasteurella pneumotropica or Corynebacterium kutscheri have been found in tumour preparations, and additional pathogens like, for example, Eperytrozoon coccoides and Haemobartonella muris and even protozoans like Encephalitozoon cunili can be significant for studies involving animal-to-animal passages of materials (*National Research Council* 1991).
The risk of contamination in biological materials can be reduced by in vitro-passages of tumours or by purification steps. Nevertheless, there is still a remaining risk which should not be underestimated. Especially newly arrived materials which have been in contact with rodents should be handled like animals of undefined status and should not be used in the animal house unless they have been monitored for pathogens. Meanwhile several institutions test biological materials for murine viral contamination and supply virus-free materials.

## 3. Personnel

Humans can act as mechanical or biological carriers of microorganisms. Humans are unlikely to be an appropriate host where murine pathogens can reside and replicate, but several human microorganisms can cause infections in rodents, at least in immunosuppressed animals.
It has to be assumed that each microorganism which is present in humans who have access to a barrier unit might sooner or later colonize the animals because common housing systems allow contact between humans and animals. *Bacteria of human origin* like Escherichia coli (*Thunert* 1978) or Staphylococcus sp. (*Lenz et al.* 1978, *Wullenweber-Schmidt et al.* 1987) can therefore regularly be isolated from rodents. They are acceptable for most of the animal experiments if problems do not become obvious. Transmission can for certainty not be avoided in barrier-reared colonies, not even by gloves or surgical masks.
Some microorganisms of human origin like Staphylococcus aureus or Klebsiella pneumoniae are occasionally responsible for health problems or research complications, mainly in immunocompromised animals. In such cases, transmission from humans to animals (or vice versa) can be avoided by establishing strict barriers between humans and animals. This can, when necessary, easiest be achieved by housing animals in isolators.
Little published information is available on the role of humans as *mechanical vectors.* There is no doubt that microorganisms can be transmitted by handling as recently described (*La Regina et al.* 1992). Microorganisms can even be transported from pets to laboratory animals by human vectors (*Tietjen* 1992). Such examples stress the need of proper personal hygiene and the importance of motivation of the staff, training and continuous information about infections and their transmission. In addition, it is an important task of the management to ensure that personnel coming into contact with animals has no access to animals of a lower microbiological quality. Laboratory staff involved with numerous projects within the same or other institutions can play an important role as vectors of microorganisms or parasite eggs. Humans are one reason why maintaining colonies of pathogen-free rodents in close association with infected animal colonies is very difficult. The importance of humans as mechanical vectors is reduced when contact to infected animals or pets is prevented.

It is sometimes recommended that personnel should not be permitted to eat or drink in barrier units. Contaminated food is unlikely an important source for rodent microorganisms. A theoretical risk of contamination can be eliminated by restriction to autoclavable food or the use of food in hygienic packing which can be desinfected by passing peracetic acid locks.

## 4. Vermin

Flying insects do not present a serious problem. Usually insect-electrocuting devices are employed in corridors, and air filters remove them from the incoming air. Crawling insects like cockroaches are more difficult to control and cannot be excluded for certainty (*Eaton* 1986). The most serious problem arises from wild rodents which are frequently carriers of infections. Modern animal houses usually have devices which prevent their entry although they can occasionally enter through very small holes. Nevertheless, animal diets, bedding or waste attract wild and escaped rodents and other animals. Areas where such materials are stored therefore need to be monitored for insect and rodent contamination (*Small* 1983). Usually the design of an animal house in combination with proper hygiene measures are able to control vermin and to reduce their importance to a minimum.

## 5. Additional factors

*Equipment* and *experimental materials* which have been in contact with animals are easily contaminated. Important factors are instruments or equipment which have to be shared with other groups. This can frequently not be avoided. In many cases compromises are necessary, especially when complicated optical or electronic instruments which cannot be sterilized are used. These are important factors which must be resolved by the person responsible for the animal quality.

Survival of infectious agents in the environment varies with temperature and humidity. Their survival time is usually limited at the climatic conditions in animal laboratories. Several bacterial species like Staphylococcus aureus, Pseudomonas aeruginosa, or Proteus mirabilis lose viability within two days at 58 % or 77 % relative humidity at room temperature (*Nicklas & Böhm* 1981). Parasitic eggs or cysts are more resistant (*MacArthur & Wood* 1978). Parvoviruses have extremely high resistance and may survive in the environment for months, and high temperatures are necessary for inactivation (*Fassolitis et al.* 1986). Nevertheless, entry or pathogenic microoranisms via *food, water or bedding* is uncommon. Drinking water is unlikely to be a source of infection. If necessary, it can be treated to eliminate infectious microorganisms. The risk of introducing pathogens by contaminated food is diminished by the heat created during the pelleting process (*Stott et al.* 1975), it can be excluded by pasteurization, radiation, or autoclaving. Bedding can easily be autoclaved or otherwise sterilized.

All *air* entering animal units should be filtered to remove dust particles and vermin. Frequently the air supply is passed through socalled HEPA filters to minimize the risk of introducing air-borne organisms as recommended by *GV-SOLAS* (1988). Such filters increase running cost, and their necessity is sometimes questioned (*Clough* 1986) because air-borne microorganisms are usually carried in aggregates or associated with other particles. *Mrozek et al.* (1991) compared different air filter sets and found out that air filters of lower efficiency (EU 9) were sufficient to maintain the status of gnotobiotic mice and to prevent transmission of MVM from experimentally infected mice to virus-free animals. The efficiency of filters chosen for a facility depends on the microbiological 'challenge' contained in the air and the status of the animals (*Clough* 1986). Basing on his experience, 5 µm filters with an efficiency of 95 % are an adequate barrier and are sufficient to prevent introduction of disease agents into pathogen-free colonies.

Spreading of microorganisms within an entity can hardly be avoided. Microorganisms are easily spread by humans carrying them on their skin or clothing. Dust is formed by animals and while cages are being changed and may contain pathogens (*Small* 1983). Therefore, the formation of dust must be reduced as far as possible. As a principal hygienic rule, it should be removed by wet cleaning with disinfectants.

*Summary*

The risk of introducing pathogens or other unwanted microorganisms into research colonies of rodents is higher than into breeding colonies. To achieve a realistic control of infectious agents, a simultaneous attack should be made on all possible routes of contamination. In this context, the importance of an adequate management system in addition to a proper design of the animal facility is stressed. Animals are considered the most important factor. As most infections in rodents are subclinical, exclusion of infection can only be achieved by appropriate diagnostic methods. Further, biological materials which have been passaged in rodents are an important source of microorganisms. They can be stored frozen for many years without any loss of infectivity. Moreover, rodents are at risk from people who frequently act as carriers when they have been in contact with infected animals or contaminated equipment. In addition, the importance of vermin and other risk factors like food, water, and air is briefly discussed.

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