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Health Monitoring of Experimental Rodent Colonies; An Overview

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Introduction

The main purpose of health monitoring in laboratory animal units is to avoid infections which might influence the health or physiological characteristics of the animals. Appropriate health monitoring helps to avoid imprecise results and enables the performance of essential experiments with a minimum number of animals. Detection and subsequent elimination of disease factors is therefore a means to get better and more reliable results in animal experiments. The use of standardized animals is a basis for the reduction of the number of animals used and thus makes an important contribution to animal welfare.

It is not possible to devise one health monitoring programme which will be applicable for all colonies of laboratory animals. Each animal facility has its own requirements, types of experiments, different animal species, and traditions. The aim of this paper is, therefore, to present some suggestions which might suit a multipurpose animal facility. It will focus on rats and mice as the most commonly used laboratory species. Some of the points have been discussed within the FELASA working group on animal health which is preparing recommendations for health monitoring of rodent and rabbit experimental units (*Rehbinder et al.*, in press).

Three different groups of personnel are involved in an efficient monitoring programme and need to interact. The first group is the personnel in the diagnostic laboratory who are, nevertheless, dependent on the personnel responsible for the animal facility. This latter group is central to the whole programme. They are responsible for the effective implementation of the monitoring programme, including sampling. They should consider not only their own interests in health control, but also the needs of the experiments. They have to convince researchers that good research requires microbiologically standardized animals. The third group, the researchers must support the monitoring programme although it may cause time delays, cost money and complicate the availability of animals.

The aim of health monitoring

Animals used in scientific experiments should be free from infectious agents which might influence their health (and the health of humans) and their response in experiments. Most infections are subclinical but can nevertheless modify research results (for details, see *Bhatt et al.* 1986, *National Research Council*, 1991). Therefore, detection of the presence of infection, whether or not it causes clinical disease, is necessary. Monitoring must include animals in the colony and risk factors such as biological samples which may be introduced into the colony.

Most monitoring programmes will primarily focus on infectious agents. It is usually not the aim of a health monitoring programme to define physiological characteristics (e.g. normal values of liver enzymes, CD-antigens, lymphocyte populations, IgG subclasses for specific mouse strains, tumour prevalence) although these factors might be important for proper performance of experiments. Most facilities will not have the capacity to perform such important, but very specific investigations as a service on a routine basis. Monitoring of these parameters is usually part of the research project und will not be discussed here.

The monitoring programme

Each monitoring programme will be tailored to the conditions it must serve. It is therefore influenced by various characteristics of the institution. Most importantly, it is dependent on the research objectives but in addition on the physical conditions and layout of the animal house, the husbandry methods used and the sources of animals introduced. The pro-

gramme is further influenced by the staffing levels (quality as well as number of personnel), the available diagnostic laboratory support and finances. It may be desirable in a multipurpose unit to have a range of monitoring programmes (e.g. for isolator-housed versus barrier-housed animals).

A range of important factors may impact the health of animals or the outcome of experiments. Among them are the quality of animals, biological samples and other materials necessary for the research (e.g. chemicals, drugs, instruments, plastic or glass containers). Also materials associated with the animal housing(e.g. bedding, food, water, air, cages and drinking bottles). Each factor can be a potential source of infectious microorganisms. Some might be contaminated with mycotoxins, insecticides, metal residues or dust particles. It is obvious that not all the factors which might influence the outcome of animal experiments can be included in a routine monitoring programme.

Furthermore, not all available test methods can be used routinely. For example, histopathology might not normally be performed. Researchers using a variety of transgenic strains are seeking the answers to specific questions such as the function of a gene or consequences of lack of a gene. There may not be the resources centrally to test for morphological abnormalities which are specific for a certain small population and not relevant to the whole unit.

A health monitoring programme will primarily cover those factors which are most likely to influence either the animals directly or the experiments. Health monitoring of mice and rats on a routine basis will mainly focus on infectious agents. This is realistic and will in many cases be sufficient. However, there are examples which show that non-infectious factors like the quality of drinking water, bedding, light, noise, and nutrition will have an impact on animal health. Quality assurance of these factors becomes necessary in specific cases. For example, problems have been reported with drinking water acidification systems which result in increased concentrations of chromium and nickel. Under such circumstances, it has been necessary to routinely monitor for metal ions in the drinking water until the problem had been resolved.

Difference between breeding and experimental colonies

The risk of introducing infections into experimental colonies is much higher than in breeding colonies. Major sources of infections are:

– *Animals.*

In many experimental colonies, a variety of strains, including congenic and transgenic strains, from various breeding units are introduced on a regular basis.

– *Experimental materials.*

Often materials and samples are needed which cannot be disinfected. A high risk of introducing pathogens arises from biological materials like cells, sera, tumours and ascitic fluid which may derive from infected animals or humans and may therefore be contaminated. The importance of biological materials as risk factors is very often underestimated.

– *Personnel.*

Compared to breeding units, more personnel must have access to their animals. Often, scientific personnel are also more difficult to control than animal technicians.

What should be monitored?

1. Animals

The monitoring programme must focus on the most important risk factors (Nicklas 1993). Special care is necessary for the surveillance of animals since they are most likely to carry microorganisms which are relevant or specific for their species and therefore present the greatest risk. More details must be considered concerning animals.

1.1. Origin of animals

Sources can be monitored to get information on unwanted microorganisms or other factors within a supplying unit. Thus, quality control can have the function of an early warning system. Monitoring animals from external sources aims at timely detection of unwanted agents in order to prevent their introdiction. Animals from external sources can be of variable quality. They will in many cases be available with a reliable health certificate demonstrating that they are free from infectious agents. Most animal facilities buy such animals from commercial breeders. It might be possible to introduce such animals into a holding area before results of monitoring or rechecking are available. The risk of introducing pathogens may be acceptable when direct introduction is restricted to animals coming from sources of well-known microbiological status. Nev-

ertheless, such animals deserve attention and should be checked to verify the breeder's results. Even if it is impossible to check a statistically valid sample, it has some advantages. Most of all, it will encourage the supplier to monitor his colonies and communicate results honestly. Animals of unknown status coming from external sources should be regarded as being infected unless their status has been defined. Like animals known to harbour unwanted microorganisms, such animals must be kept isolated from other animals. In many cases it will be reasonable to keep them in permanent isolation.

1.2. Strain/stock of animals

The strain of animals submitted to monitoring is dependent on the aim of the monitoring. If possible, animals of the same strain or stock as used in experiments should be used for monitoring. However, this is usually not essential for serological and bacteriological testing though there are numerous examples of strain-specific reactions. For example, Streptobacillus moniliformis infects mainly C57BL6 mice, mice of other common strains are usually not susceptible to infection and do not develop antibodies (*Wullenweber et al.* 1990). Strain susceptibilities for Mycoplasma pulmonis and many other microorganisms have also been reported. This problem of strain specificities is most obvious in immunodeficient animals like nude mice which do not produce sufficient amounts of antibodies. In such cases, immunocompetent sentinel animals must be used for serological monitoring. On the other hand, immunodeficient or immunosuppressed animals may be very useful to monitor for bacterial or parasitic infections. The strain can be very important when clinical disease or histopathological changes are seen. For example, C57BL6 mice are relatively resistent to ectromelia virus. Infections in this strain are often inapparent whereas DBA/2, CBA, BALB/c and other strains show severe clinical disease. In contrast, C57BL6 mice are relatively susceptible to mouse hepatitis virus (MHV), whereas other strains (eg A/J) are resistant (*Kunstyr* 1992).

1.3 Age

For breeding colonies, detailed recommendations exist (*Kraft et al.* 1994). In experimental colonies, it may be difficult to follow strict rules concerning the age of animals because animals of predetermined ages will not always be available for monitoring. When animals in an experimental unit are exclusively purchased, the time they have been in the unit is more important for monitoring than their age. In general, young animals or animals that have been in a colony for a short time provide a current picture of the colony status whereas long-term residents give a historical picture. If colony-derived animals are not available, sentinels are equally useful. They should be tested after a minimum of 4-6 weeks in the facility.

1.4. Frequency

In many cases the frequency of monitoring will depend on various factors like the animals' value, the risk of introducing microorganisms, the status, etc.. Many facilities test quarterly as recommended in the FELASA recommendations for breeding colonies (*Kraft et al.* 1994). In most multipurpose animal units more frequent monitoring is preferable as it will result in earlier detection of an infection. As a general rule, it is therefore recommended to monitor about 3 to 5 animals from each animal room every four to six weeks instead of 10 from a whole unit every three months. Naturally, additional monitoring is necessary if suspicion of an infection arises between scheduled testing dates.

1.5. Number of animals

The number of animals recommended to be monitored is dependent on many characteristics of the colony. Different sample sizes are necessary for animals housed conventionally versus in microisolator cages due to different characteristics of spread of an infection. The goal is to detect the presence of an organism in at least one animal. A formula exists which aids to calculate the sample size for a desired probability of detection (*ILAR Committee on Long Term Holding of Laboratory Rodents,* 1976). The sample size is often also a compromise between such calculations, and financial factors. Animals must normally be tested individually. It may be preferable to monitor a small number of animals frequently rather than a large number say once a year. In the same way, small numbers from many locations should be sampled in preference to large numbers from only one or a few locations. Independent from the number of animals which is scheduled for routine monitoring, animals with clinical disease should be submitted for direct examination for microorganisms (bacteria, parasites, viruses) and for histopathology.

1.6. Monitoring units or experiments

It might be advantageous in some experimental units to monitor animals in correlation to specific experiments. However, in many universities or multipurpose research institutes, different experiments are commonly performed in one unit. In such cases it is easier and more useful to monitor the status within a unit irrespective of the experiment, and to make such data available to all investigators who are responsible for experiments in this unit. This is the same as in breeding units where results obtained from random samples are supposed to be valid for all animals of the same species within a barrier unit, irrespective of the strain, age, and other variables.

1.7. Sentinels

A sentinel animal is an animal obtained from a breeding colony of known health status which is placed among animals of the same species to aid in evaluation of the new colony status. Sentinels are often used in experimental colonies when experimental animals are not available for monitoring. It is important that sentinels are strategically placed within an animal room. They should be housed in a way that they receive maximum exposure to potential infections. They should be housed in specific cages in various locations within an animal room. The cages should be placed on the bottom shelves without filter tops so that they receive debris from animals above to increase exposure. In addition, contact between sentinels and experimental animals can be increased by housing sentinels in cages which contain soiled bedding from different locations within the room. Each time the cages are changed, soiled bedding is transferred to sentinel cages. Introduction of sentinels into a unit is always, at least theoretically, connected with a certain risk. Therefore in long term experiments sufficient numbers of animals should be housed with the experimental animals from the outset to guarantee that the minimal sample size will be available throughout the whole period of the experiment. When short-term experiments are performed or in multipurpose units, the unit can be restocked repeatedly. In this case, sentinels removed for monitoring can easily be replaced during restocking with experimental animals from time to time.

Table 1. Contamination of transplantable tumours with murine viruses.

Origin of tumours	Propagation	No. monitored	No. negative	No. positive	% positive
Mouse	in vivo	83	26	57	68.7
	in vitro	86	79	7	8.1
	total	169	104	64	37.9
Rat	in vivo	45	43	2	4.4
	in vitro	23	23	0	0.0
	total	68	66	2	2.9
Human[a]	in vivo[b]	45	40	5	11.1
	in vitro	72	71	1	1.9
	total	117	111	6	5.1
Hamster	in vivo	14	10	4	28.6
Rabbit	in vivo	1	1	0	0.0
Total		**369**	**292**	**76**	**20.6**

[a]Each human tumour tested had been passaged in nude mice.
[b]Specimens monitored in the MAP test were propagated in nude mice.

1.8. Quarantine

There is no doubt that infected or diseased animals or animals of unknown microbiological status must be kept isolated from other animals. However, in many cases this is not possible for all animals brought into an experimental unit. Then, a quarantine period is often recommended (*Thunert et al.* 1988) during which monitoring can detect historical infections. Animals should be observed for clinical signs of disease they may be incubating.

2. Biological materials

Another complex which should be included in a comprehensive monitoring programme are biological materials. They may derive from infected animals and therefore be an important source of unwanted microorganisms which could infect animals. Due to the high risk of introducing microorganisms by biological materials (*Collins & Parker* 1972, *Nicklas et al.* 1993), they should be demonstrated to be free of pathogens before being introduced into an animal unit. Each sample of transplantable tumour or unpurified ascitic fluid should be monitored by culture for bacteria and fungi. In addition MAP-testing should be performed to detect viral contamination. Table 1 shows current data from our laboratory which demonstrates the high contamination rate of biological samples. The most frequently detected virus is lactate dehydrogenase-elevating virus (LDV) which is highly significant when passaged in mice and in research involving transplantable tumours, viral oncology, and immunology (Table 2). The problem of transmitting murine pathogens through tumours can largely be avoided by using pretested tumour lines which are available from tumour repositories.

Infectious agents which should monitored

Lists of infectious agents to be monitored have been published by various organisations (*eg Kunstyr* 1988). In this publication over 20 viruses, several mycoplasma species, about 25 bacterial pathogens, several bartonellas, fungi, spirochaetes, protozoans, helminths and about a dozen species of arthropods are listed which may be of importance in colonies of rats and mice. Monitoring on a routine basis for each of the organisms mentioned is neither realistic or necessary. The most important micororganisms are those that pose a threat to research or to the health of animals and humans and, in addition, those we can hope to eliminate. That means that it is acceptable, for example, to exclude oncogenic retroviruses. Other microorganisms may be less important because they are unlikely to occur in good-quality rodents due to repeated sanitation procedures (e. g. Brucella, Erysipelothrix, and others). Most cestodes are unlikely to be found because they require an intermediate host. A decision has to be made in each facility which microorganisms are tolerable or unacceptable. This list is again dependent on the type of

Table 2. Some known effects of lactate dehydrogenase-elevating virus (LDV) on experimental animals or on animal experiments.

1. Immunological effects:
Thymus involution; splenomegalia and lymphnode enlargement; enhancement or suppression of humoral immunity; depression of cellular immunity; changes in T cells, B cells and macrophages; interferon production

2. Endocrinological alterations:
2-10-fold increases in plasma corticosterone levels during the acute phase of infection.

3. Enzyme changes:
two- to hundredfold increases in plasma-LDH and various other plasma-enzymes.

4. Alteration of clearance capabilities:
decrease in the clearance capacities for enzymes, increase of their half-life time

5. Modifications of tumour therapy:
Potentiation of enzyme effectiveness (asparaginase, glutaminase)

6. Changes in tumour development:
increases in the incidence and growth-rate, reduction of the survival time, rejection of transplanted tumours

7. Interaction with oncogenic viruses:
suppression of the incidence of mouse mammary tumour virus, alterations in oncogenic viral expression of Moloney sarkom virus, Rauscher leukemia virus

research for which the animals are used. For example, Staphylococcus aureus causes serious wound infections in surgically prepared nude mice whereas there are no problems with other apathogenic Staphylococcus species like Staphylococcus hominis or Staphylococcus xylosus. The same Staphylococcus aureus does not cause clinical signs in immunocompetent animals. Each institute should prepare a list of those organisms which are not acceptable in the colony or in parts of it. Some publications can be used for guidance (*Kunstyr* 1988, *Kraft et al.* 1994). In practice, such lists do not differ much between different facilities. Highly pathogenic agents and zoonotic agents are clearly unacceptable. Testing for ectromelia, LCM, or salmonella may be given high priority. The urgency for testing however depends on the confidence one has in the source of animals. This means that it may be unnecessary to monitor animals from a reliable vendor for ectromelia, whereas animals from a dubious source should always be tested.

Methods and tests to be used

A major consideration is confidence in the testing laboratory since different laboratories may produce different results. It is easier to have confidence in a result when multiple criteria are used for the detection and confirmation of an infection. The risk of getting false-positive or false-negative results can be minimized when agent isolation, serology, pathology and other criteria are used together. This is in many cases necessary as tests vary in specificity and sensitivity from agent to agent. Testing usually starts with necropsy, followed by microscopic examination for parasites and sampling of organs for bacteriology, pathology, serology, and in rare cases for virological examination. The reliability of the results is very much dependent on experience. The use of test kits in bacteriology which have been developed for bacteria of human origin may lead to false results. For example, the mouse-specific bacterium called Citrobacter freundii 4280 is not correctly identified with the commercially available API system when the typical profile of this organism is used to obtain an identification with the APILAB PLUS computerized identification system. Similar problems exist with bacteria of the Pasteurella-Actinobacillus-Haemophilus group. Therefore, such investigations should be performed in laboratoriess with sufficient expertise in microbiology of the relevant species. Serological tests also require technical competence to ensure sufficient standardization of reagents (including controls) and accurate interpretation of results. Serological results are much influenced by the test used. When the highly sensitive ELISA or indirect immunofluorescence (IIF) methods were used instead of the more specific haemagglutination inhibition (HAI) test, antibodies to minute virus of mice (MVM) or Kilham rat virus (KRV) were detected in formerly negative colonies. It transpired that these reactions were caused by cross-reactivity with other parvoviruses. The complement fixation (CF) test is relatively insensitive but very specific for antibodies to specific strains of mouse hepatitis virus (MHV). For that reason, mixed antigens containing different virus strains must be used to detect from the broad panel of different types of MHV. ELISA or IIF are more sensitive but less specific, and most strains will cross-react. Therefore, these latter tests are more reliable in the case of rodent coronaviruses. Mycoplasma serology is easy to perform but is not always accurate because of inadequate specificity and low antibody levels. Mycoplasma culture is very time consuming. Serological testing is useful as a first step to get an indication of a Mycoplasma infection. Confirmation should be by culture of lungs, trachea and nasopharynx and subsequent serological characterization. Serological tests are indirect tests that rely on antibody responses. Therefore, serological results based on a single serological test are of less diagnostic value than positive results in a direct test such as isolation and identification of a microorganism. As false-positive or false-negative serological results can easily occur, testing strategy should rely on a primary test for each agent with one or several confirmatory tests. Further, it may be advisable to confirm in another laboratory. In general, serology is a reliable approach to detect viral infections within a population. However, seroconversion only signifies previous contact with virus and does not indicate if virus is still present. Serology cannot detect a viral infection during the early, acute phase. During the acute phase of an infection, histopathology in combination with immunohistochemistry may therefore give more reliable results.

The Health Status Report

The health report should contain sufficient information to provide a reliable indication of the quality of the animals and be a basis for decisions. Single animals are monitored, but cumulative information on

the status of the whole population should be given in the health report sheet. Unfortunately, each animal facility or breeder has his own style of report sheets. Often, microorganisms which are present in a colony are not listed and only those which are not present are mentioned. Some health reports are therefore difficult to read and interpret. For this reason, a FELASA working group (*Kraft et al.* 1994) recommends to use a uniform health report for breeding colonies. A similar health report should be used for experimental colonies. Some additional information might be reasonable (e. g. housing conditions, treatment) and should be included. Table 3 gives a checklist of minimal information which should be included in a health status report.

Table 3: Minimal information which should be given in a health report.

Some minimal information should be given in a status report when animals are shipped to external colonies. These are:

- exact location (designation) of the colony
- housing conditions (conventional, barrier, isolator)
- name(s) of laboratory(ies) involved in monitoring
- date of restocking/rederivation of the colony
- date of last monitoring
- no of animals monitored since date of restocking or during the last twelve months
- methods used (clinical signs, microscopy, microbiological culture, serology, etc.)
- name(s) of pathogens detected in the colony
- name(s) of pathogens not detected in the colony
- treatment, vaccination, etc.

Detailed results of the last monitoring should be added.

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