Animal Experiments in Medicine

Animal houses, breeding of experimental animals

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An experimental animal can serve as a model for a human being in all those functions in which there is a considerable degree of genetic similarity. The experiments can be acceptable for everybody only if the results are unquestionable and reproducible, i.e. exact. This can be the case only if the experimental (treatment) and control groups differ in only one aspect, i.e. the aspect under investigation. There are significant differences in sensitivity between species because of their differing genetic backgrounds. This is the reason why different species are appropriate or not for a given experiment.  

An important requirement for laboratory animals (apart from price) is that they should be easy to keep. Today, rodents meet this demand best. Of all the mammals, mice and rats have been most frequently used in laboratories (approximately 90%), but in the last 20 years the proportion has decreased to 1/10. As compared with these species, the number of animals that can be kept as pets, such as guinea pigs, rabbits and dogs, is very low. The reasons for this are the increasing frequency of experiments without the use of animals, or the use of animals with particular characteristics (e.g. inbred, SPF, see below), and the rising prices.  

1. Biology of the most frequently used mammals  
1.1. Mouse  
Seventy per cent of all animals used in biomedical activities are mice. More than one thousand stocks and strains of mice have been developed, as have hundreds of mutant stocks that are used as models of human diseases. In terms of genetics, the mouse is the most thoroughly characterized mammal. The life span is 1.5-3 years. Mice are very active animals. They are sensitive to changes in temperature, draughts and high humidity. The incisors are open-rooted and grow continuously. Mice will bite or "pinch" with their sharp incisors if mishandled. Mice should be fed a commercial pelleted mouse or rodent diet and water ad libitum. Mice are polyestrous animals and breed the year round; ovulation is spontaneous. A fertile postpartum estrus occurs 14-24 h following parturition. The duration of the estrous cycle is 4-5 days. The average gestation period is 20 days. The average litter size is 10. The pups are weaned at 3 weeks of age.  

1.2. Rat  
The rat is the second most commonly used animal in biomedical activities, surpassed only by its relative, the mouse. Various stocks, strains and mutants are available, but much fewer than for the mouse. Most laboratory rats are outbred albinos. Rats are less active and aggressive than mice. They are typical rodents, with continuously growing incisors. Rats should be fed a commercial pelleted rat or rodent diet and water ad libitum. The life span is 2.5-3.5 years. They are sensitive to draughts, a high level of ammonia, low humidity and noise. They are susceptible to respiratory diseases. Rats too are polyestrous animals and breed the year round; ovulation is spontaneous. The duration of the estrous cycle is 4-5 days. The average gestation period is 22 days. A fertile postpartum estrus occurs within 48 h of parturition. The average litter size is 6-12. The pups are weaned at 3 weeks of age.  

1.3. Hamster  
Hamsters are the third most commonly used research animal. Ninety per cent of them are Syrian hamsters. Hamsters have a tendency to bite if roughly or improperly handled, startled, injured or awakened. Females tend to be larger and more aggressive than males. The life span is 1.5-2 years. The incisors are open-rooted and grow continuously. The cheek pouches of the hamster extend to the scapulae and can be everted. Hamsters should be fed commercial pelleted hamster or rodent diet and water ad libitum. The flank or scent glands
appear as dark patches on either flank. They are sebaceous glands that function in marking territory and mating behavior. The urethra of the female exists separately just ventral to the vulva. Breeding onset is at approximately 90 days. Hamsters are polyestrous animals and breed the year round; ovulation is spontaneous. The duration of the estrous cycle is 4 days. The average gestation period is 16 days. The average litter size is 5-9. Cannibalism is more common with hamsters than with other laboratory rodents. The young are weaned at 21 days.

1.4. Guinea pig  
The most common stocks used in research are shorthairs. Guinea pigs panic easily in response to a new or frightening experience, scattering to the corner of the cage or freezing in place. Teeth-chattering of nervous guinea pigs can also be seen. The life span is 4-5 years. All teeth are open-rooted and grow continuously. The guinea pig has a large cecum. Guinea pigs have a dietary requirement for vitamin C, and should therefore be fed a commercial pelleted diet formulated specifically for guinea pigs. Guinea pigs are polyestrous and breed the year round; ovulation is spontaneous. The duration of the estrous cycle is 15-17 days and estrus itself lasts 24-48 h. The average gestation period is 65 days. The average litter size is 3-4. The young are precocious at birth 3-4; weaning occurs at 14-28 days.

1.5. Rabbit  
The breeds of rabbit most commonly used in research are the New Zealand White and the Californian, which weigh 2-5 kg, i.e. they are large breeds. The life span is 5-6 years. All the teeth are open-rooted and grow continuously. The rabbit cannot vomit, like the rat and horse. The cecum is large, and terminates in the vermiform process or cecal appendix. This appendix contains a large amount of lymphoid tissue. Rabbits are coprophagous. They consume soft, moist fecal pellets produced at night (night stool). Rabbits should be fed a commercial pelleted rabbit diet; water should be provided ad libitum. Diets such as these are nutritionally complete and do not require supplementation. The highly vascularized ears are important in thermoregulation. The uterus is duplex with separate cervical and uterine openings. Breeding onset in the medium-sized breeds is at the age of 5-6 months. Rabbits are induced ovulators with no regular estrus cycle. The vulva of the receptive doe is congested and purplish. The average gestation period is 32 days. Shortly prior to parturition (kindling), the doe builds a nest, pulling large amounts of hair from her mammary region and forelimbs. The average litter size is 7-8. Weaning occurs at 4-6 weeks.

1.6. Pig (potbellied pig, micropig, minipig)  
Pigs are social animals and have a rigid dominance hierarchy. If pigs are group-housed, they will generally fight for the first 24-48 h to establish dominance, which is almost directly related to size. Pigs in general are friendly and docile, but will react severely to poor handling or a stressful environment. The life span of the potbellied pig (PBP) is probably 8-20 years with ~10-15 years typical. The PBP is sensitive to extremes of heat and cold and should be provided with a clean, dry, draught-free environment. Extended exposure to high temperatures combined with high humidity may be fatal to the PBP.

1.7. Cat  
Cats are strict carnivores and predators. They have excellent vision and hearing, but their sense of smell is not as well developed as in the dog. Cats are generally solitary by nature, with strong territorial ties. At most they may form loose-knit social groups. Territories are marked by urine spraying and smearing surfaces with the scent glands located in the chin
and in the head, in front of the ears. Cats are generally non-social, but can adapt to group living. An ideal group is approximately 20 individuals, as this enables a hierarchy to form which tends to be relatively stable. Cats have certain physiological features that are more in common with those in humans than the laboratory rabbit or rodent; hence, they have been extensively used in behavioral and biomedical research, particularly in the neurological sciences. Bred cats can generally live for 10 to 13 years. Cats cannot survive on diets adequate for other carnivores, unlike dogs (e.g. cats require taurine). The female cat (or queen) is seasonally polyestrous, but a small percentage can breed continuously. Queens can be run as a harem-mating system. The only way to detect estrus is via the behavior changes (and the vaginal smear). Estrus behavior lasts for 4-6 days. The queen is an induced ovulator. The gestation period is 59-65 days. The average litter size is 4-6 kittens. The kittens are generally weaned at 6-7 weeks.

1.8. Dog
The domestic dog is a species with a large number of breeds rather than genetically defined strains. There are marked differences between the various breeds in size, appearance and life expectancy. Dogs are highly social, smart, intelligent animals. Both nonspecific and human social contacts are extremely important for their well-being. Their social isolation and solitary housing are therefore considered to be important stressors. They do best when housed either in pairs or in small compatible groups in an environment affording some level of complexity and choice. Daily contact with caretakers is important. The dog has been used as a model of many human conditions in areas such as cardiovascular research, pharmacology and toxicology. The average life span is 8-12 years. Dogs used for research or teaching may be purpose-bred. The typical laboratory dog is the beagle, but other breeds are also used in large numbers. Dogs are carnivores with monogastric digestive systems. They should be fed only complete and balanced diets. Many commercially available dog foods contain all essential nutrients in their required proportions. These foods are manufactured in dry, semi-moist and canned forms. Reproductive data: the age of puberty is 7-10 months. The length of the estrus cycle (ovarian cycle) is 21-28 days. In most breeds the interval between estrus periods is 6-7 months. Females (bitches) ovulate spontaneously. The average length of gestation (pregnancy) is 63 days. The average litter size is 3-8. The puppies are generally weaned at 6-7 weeks.

2. Housing of experimental animals
2.1. General principles
Proper housing and good management of animal facilities are essential for the animal well-being, the quality of the research data, and the health and safety of the personnel. At a minimum, an animal must have sufficient space to turn around and to express normal postural adjustments, it must have ready access to food and water, and it must have enough clean-bedded or unobstructed area in which to move and rest. Animals should have opportunities to exhibit species-typical activity patterns. Whenever appropriate, social animals should be housed in pairs or groups, rather than individually, provided that such housing is not contraindicated by the protocol in question and does not pose an undue risk to the animals. Highly social and many other domesticated animals benefit from positive human interaction.

2.2. Physical facilities
The microenvironment of an animal is the physical environment immediately surrounding it (the primary enclosure), with its own specific factors (such as a cage or pen).
The primary enclosure provides the limits of the animal’s immediate environment. The macroenvironment is the physical environment of the secondary enclosure (such as an animal holding room, a barn, etc.). Basic types of establishments for animal keeping are breeding / supply establishments and user establishments. Important elements of the primary and secondary enclosures are as follows:

- A cage is a permanently fixed or movable container that is enclosed by solid walls and, at least on one side, by bars or meshed wire, in which one or more animals are kept or transported; depending on the size of the container and the stocking density, the freedom of movement of the animals is relatively restricted.

- A pen is an area enclosed by walls, bars or meshed wire, in which one or more animals are kept; depending on the size of the enclosure and the stocking density, the freedom of movement of the animals is usually less restricted than in a cage; pens must be designed for the well-being of the species; they must permit the satisfaction of certain ethological needs (for example, the need to climb, hide or shelter temporarily).

- A run is an area enclosed by walls, fences and bars or meshed wire and frequently situated outside permanently fixed buildings, in which animals kept in cages or pens can move freely during certain periods of time.

- An animal holding room is a room situated inside a building where animals are housed in cages or pens, either for breeding and stocking, or during the conductance of an experiment.

The majority of holding rooms are usually designed to house rodents. Such rooms may also frequently be used to house larger species. Care should be taken not to house together species which are incompatible. Walls and floors should be smooth, impervious and have a non-slippery, easily washable surface. Drains, if any, should be adequately covered. Such rooms should be provided with facilities for carrying out minor experiments and manipulations, where appropriate.

Service rooms are rooms for the storage of food, clean cages, bedding, cleaning and washing materials. The rooms for food and bedding must be cool and dry, and vermin- and insect-proof. The rooms for clean cages, instruments and other equipment must be adequately designed; the cleaning process must be arranged so as to separate the flow of clean and dry equipment in order to prevent the contamination of newly cleaned equipment. 1,2,4

2.3. Housing of the most frequently used species

**Rodents, guinea pig, ferret**

These animals are usually kept in plastic cages with solid bottom and walls, of different sizes (shoe-boxes), with roofs made of wire, and with bedding. Guinea pigs and ferrets may be housed in small pens, made of plastic, metal or concrete, enclosed by low walls, with open roof and with bedding (wood shavings). Ferrets and guinea pigs are social animals, and it is therefore best to keep them in pairs or in groups. They need a complex, dynamic environment. For guinea pigs, it is necessary to provide a shelter in their cage or pen, where they can seek safety.

**Rabbit**

Rabbits are usually kept individually in small cages with a solid or meshed-wire bottom. They are gregarious animals, and should therefore be housed in compatible groups in pens with bedding (indoor or indoor-outdoor).

**Cat**

Cats are kept in individual caging, or in group pens or cages. The housing of cats in cages should be strictly limited. They should be let out for exercising at least once a day, if this does
not interfere with the experiment. In the group situation, the most essential criterion for acceptable housing is the provision of an adequate number of hides and escapes for cats. Cat pens should be equipped with dirt trays, an amply raised shelf room for resting, and objects suitable for climbing and claw-trimming. In a closed pen, many non-convertible windows are desirable.

**Dog, swine (miniature pig)**

There is a great range of possibilities as concerns housing: it may be situated in indoor or outdoor pen areas, with open access to the outside run, or in an indoor environment with some sort of complexity built into it. Single housing may be necessary because of the demands of an experiment. Dogs can be given opportunities for activity by having access to a run or being moved into another area (such as a large cage or outdoor pen), which provides more space for movement.

**Monkeys (non human primates)**

Non human primates are social animals which, when deprived of companionship for an extended time, develop unmistakable signs of depression and frustration (as do dogs and pigs). They are physiologically and anatomically adapted to live in a complex, dynamic environment. Housing such animals in pairs offers a practicable alternative to group-housing. Raised resting surfaces or perches are also desirable for monkeys and dogs.

3. **Husbandry**

The person in charge of the establishment must ensure regular inspection of the animals and supervision of the accommodation by a veterinarian (or other competent person).

3.1. **Food, drinking water and bedding**

**Feed**

Animals should be fed palatable, non-contaminated and nutritionally adequate food, daily or according to their particular requirements, unless the protocol in which they are being used requires otherwise. Nowadays, the feed is ‘standard’ mixed feed, pressed into pellets for almost all species (dry laboratory-animal diets). Packing, transport and storage must be such as to avoid contamination, deterioration or destruction. The major problems with feed may be an increase in the number of bacteria, rancidity, and the appearance of toxins caused by fungi.

The feed distribution process may vary according to the species, but it must be such as to satisfy the physiological needs of the animal: for rodents *ad libitum* (as much as they like), but for cats, dogs and pigs in portions. Feeders should be designed and placed so as to allow easy access to food and to minimize contamination.¹⁶

**Drinking water**

Animals should have access to potable, uncontaminated drinking water according to their particular requirements. (During transport, it is acceptable to provide water as part of a moist diet.) Watering devices should be checked daily to ensure their proper maintenance, cleanliness and operation. The methods commonly used in watering involve bottles, dishes and automatic systems. Bottles are often used for small animals such as rodents and rabbits, while dishes are customary for cats, dogs or pigs. When bottles are used, they must be made from translucent material in order to allow their contents to be monitored. All bottles and accessories must be taken to pieces, cleaned and sterilized at appropriate and regular periods. It is better to replace water bottles than to refill them, because of the potential for microbiologic cross-contamination.
Bedding

Bedding must be dry, absorbent, non-dusty, non-toxic and free from infectious agents or vermin or any other form of contamination. Ordinarily, softwood shavings and chips are appropriate and used, made from non resinous wood. Special care must be taken to avoid sawdust or bedding material derived from wood which has been treated chemically. Bedding should be used in amounts sufficient to keep animals dry between cage changes.

Sanitation (cleaning)

Sanitation (the maintenance of conditions conducive to health) involves bedding changing (as appropriate), cleaning and disinfection. Cleaning removes excessive amounts of dirt and debris, and disinfection reduces or eliminates unacceptable concentrations of microorganisms. The standard of a facility depends very much on good hygiene. Clear instructions must be given for the changing of bedding in cages and pens, and adequate routines must be established for the cleaning, washing and decontamination of cages and accessories, bottles and other equipment. All components of the animal facility, including animal rooms and support areas, should be cleaned regularly and disinfected as appropriate to the circumstances.

Contacts with the animals

The performance of an animal during an experiment depends very much on its confidence in man, something which has to be developed. It is therefore recommended that frequent contact should be maintained so that the animals become familiar with human presence and activity (adaptation). The staff must be sympathetic, gentle and firm when associating with the animals.

4. Factors in the macroenvironment

Temperature

The temperature must be measured continuously. The recommended (optimal) temperature range for mice, rats, guinea pigs and miniature pigs is 20–24 °C, while for dogs, cats and rabbits it is 15–21 °C. Some conditions might require an increased environmental temperature, such as postoperative recovery, the housing of some hairless rodents and the housing of neonates.

Humidity

Extreme variations in relative humidity (RH) have an adverse effect on the health and well-being of animals. The RH must ordinarily be maintained at 55±10%. Levels below 40% or above 70% for a prolonged period must be avoided.

Air ventilation

The purposes of ventilation are to supply adequate oxygen (fresh air); to remove thermal loads caused by animal respiration, lights and equipment; to dilute gaseous and particulate contaminants (e.g. ammonia or dust); to adjust the moisture content of the room air; and, where appropriate, to create static-pressure differentials between adjoining spaces. The air in the room should be changed at frequent intervals. A ventilation rate of 15–20 fresh-air changes per hour is normally adequate. The use of recycled air to ventilate animal rooms must be avoided. The ventilation system must be designed so as to avoid harmful draughts. The air used for ventilation should be heated in winter and cooled in summer. Frequent bedding changes and cage-cleaning, coupled with husbandry practices, such as a low animal
density within the room, and lower environmental temperature and humidity, can also reduce the concentration of toxic or odor-causing gases in the animal room air.\textsuperscript{1-6}

5. Microbiological environment (microorganisms and infections)

Infectious agents may affect animal populations in various ways. Some are pathogenic and may induce clinical signs with variable morbidity and mortality. However, most microorganisms induce no or only mild disease. Silent, asymptomatic infections (adventitious pathogens and opportunistic organisms) are often activated by experimental procedures (stress, immunosuppressant, \textit{etc.}) or environmental influences (transportation, toxic gases, \textit{etc.}). Frequently, certain strains of a given species are more sensitive to an infection, whereas the same agent may cause only milder or different symptoms in other strains, or the infection may be asymptomatic.

The types of organisms that can infect (experimental) animals include bacteria, protozoa, fungi, viruses, helminthes and arthropods. Many agents (even in the event of clinically silent infections) may exert impacts on the physiological parameters and hence on the results of animal experiments. It is obvious that experimental data obtained from diseased animals should, if at all, be used only with maximal caution. The use of laboratory animals that are free from unwanted microorganisms is an important prerequisite for the acquisition of reliable and reproducible results with a minimum of animals and is a significant contribution to animal welfare.\textsuperscript{1}

5.1. Microbiological quality and classifications of experimental animals

The presence of unwanted microorganisms and the suitability of an animal population for a specific experiment can be demonstrated only by comprehensive health monitoring before and during experimentation. The quality of animals is most commonly characterized in terms of the microbiological (hygienic) status and of the system used in raising animals to ensure that a specific microbiological status is maintained. There are three major types of maintenance (hygienic levels):

- Isolator-maintained: The animals are kept in a sterilizable chamber with a sterilized air supply, a mechanism for introducing sterilized materials, and a series of built- in gloves.
- Barrier-maintained: The animals are bred and kept in a dedicated space, behind a barrier. For these facilities, personnel enter through a series of locks and are usually required to shower and use disinfected clothing. All equipment, supplies and conditioned air are sterilized or disinfected. Barrier facilities are designed to exclude organisms for which animals are the primary or preferred hosts, but will generally not exclude organisms for which humans are hosts. Barrier maintenance can also be achieved at the cage or rack level with equipment that can be sterilized or otherwise disinfected.
- Conventionally-maintained: The animals are raised in areas that have no special impediments to the introduction of microorganisms. This method of maintaining animals cannot ensure the stability of the microbiological status, because unwanted organisms can be introduced at any time. Micro-isolation cages are generally used to protect animals in otherwise conventional rooms.

Various classifications have been developed to define the microbiological quality of laboratory animals. The most important classifications (hygienic categories) are as follows:

- Germ-free (GF) animals that are derived by Cesarean section or embryo transfer and reared and maintained in an isolator with aseptic techniques.
Specified-pathogen-free (SPF) animals that show no evidence (usually by serology, culturing or histopathology) of the presence of particular microorganisms. An animal can be classified as SPF if it is free of one or many pathogens. Commercial suppliers have coined various terms to indicate the SPF status. All terms are related to specified organisms from which the animals are stated to be free and for which they are regularly monitored.

Virus-antibody-free (VAF) animals that are free of antibodies to specified rodent viruses. Animals might not be free of viruses other than those specified and might not be free of other microorganisms.

Conventional animals in which the microbial burden is unknown, uncontrolled or both.

Clean conventional or minimal disease (MD) animals that are maintained behind a low-security barrier and demonstrated to be free of selected pathogens. Use of this term should be avoided because of the lack of precision of its meaning.

5.2. Sources of infections / microbial contamination
The sources of microbial contamination include vermin, experimentally infected and spontaneously ill laboratory animals or their tissues or tumors, air, food, water, bedding, ancillary equipment and personnel. (The highest microbiological risk is man himself.) Good facility management practices and constant surveillance are necessary to minimize the introduction of unwanted microbes.

5.3. Zoonosis
Infectious diseases that may be transmitted from other animals, either wild or domestic, to humans, or from humans to animals, are called zoonoses (the latter is sometimes called reverse zoonosis). The list of potential zoonoses related to working with animals in research, teaching or testing is quite long, though in reality the risks are very low when the common small laboratory animal species are dealt with in the laboratory. There are a number of reasons for this low risk. Firstly, commercial suppliers of laboratory animals have done an excellent job of producing disease-free animals. Further, institutions have generally developed good occupational health and safety programs that include active veterinary monitoring and care programs. The risk of exposure to zoonotic diseases is greater for those who work with experimental animals from random sources (including cats, dogs and most livestock), and for field researchers studying wild animals in their habitat. Working with non-human primates in the laboratory is a special case because of the many zoonotic concerns.

5.4. Animal quarantine and stabilization
Whenever possible, the health status of every animal should be ascertained before it is brought into the facility. Quarantine is the separation of a newly received animal from those already in the facility until the health of the new animal has been evaluated. Effective quarantine minimizes the introduction of disease agents into established colonies and prevents the possibility of zoonoses.

The quarantine period should be of sufficient duration to allow the expression of diseases present in the incubation stages. Some or all of the following should be achieved during the quarantine and stabilization period: diagnosis, control, prevention and treatment of diseases; physiological and nutritional stabilization; and grooming to include ectoparasite control (many zoonotic agents require an arthropod vector).
The period of quarantine is in some cases laid down in the national animal health regulations. In others, it varies according to the circumstances and should be determined by a competent person, normally the veterinarian appointed by the establishment. Even when the animals are believed to be in sound health or, in the case of a higher microbiological status, after transportation (receiving), it is good husbandry for them to undergo a period of acclimatization (physiological, psychological, and nutritional stabilization) before being used in an experiment.

5.5. Symptoms, diagnosis, standard procedure for infected, sick animals
At the beginning of an illness, there are only general symptoms. It is usually too late to save the stock (group of animals) when specific signs are present. Clinical examination is difficult in some species (e.g. rodents). An animal that appears sick must be isolated immediately (in an SPF facility, it must be removed), and its surroundings must be disinfected. Apparently sick animal must be examined. In a conventional environment, isolated cases or the whole animal population can be treated.

6. Social environment and other environmental factors
The social environment usually involves physical contact and communications among members of the same species (conspecifics), although it can include non contact communication among individuals through visual, auditory and olfactory signals.

6.1. Space recommendations and group size
An animal’s space needs are complex, and considerations of only the animal’s body weight or surface area are insufficient. Space needs are given high priority in animal welfare regulations, where the minimal space needs and cage sizes are specified in detail. Some species benefit more from wall space (e.g. rodents), shelters (e.g. primates) or cage complexities (e.g. cats and primates) than from simple increases in floor space. Whenever appropriate, social animals should be housed in pairs or groups, rather than individually, provided that such housing is not contraindicated by the protocol in question and does not pose an undue risk to the animals. Space for group-housed animals should be based on the individual space needs, the behavior, the compatibility of the animals, the number of animals, and the goals of the housing situation.

6.2. Other factors
Effects between and within species (sound, olfactory and microbiological effects) should be considered. Because of these effects, all animal species should be kept (housed) in separated areas (rooms and barns). Noisy animals, such as dogs, pigs and nonhuman primates, should be housed away from quieter animals, such as rodents, rabbits and cats. Other effects involve light, color and the biological rhythm.¹,²,³

7. Transport conditions
All animals must be acquired lawfully. The use of purpose-bred research animals might be desirable and, for the most frequently used animals, is obligatory by the regulations. As a rule, vendors of purpose-bred animals regularly provide information that describes the genetic and pathogen status of their colonies or individual animals. All transportation is a stressful experience for animals and as a consequence, the physiological parameters may be seriously modified. For normalization of these modifications, rodents need at least 2 weeks, while animals with a highly developed nervous system (e.g. primates) need at least one
month. All parts of the transportation process must be carried out according to the prevailing animal health, transport and welfare regulations. All transportation of animals should be planned to minimize the transit time and the risk of zoonoses, to protect against environmental extremes, to avoid overcrowding, to provide food and water when indicated, and to protect against physical trauma. Animals must be in good health for transportation and it is the duty of the sender to ensure that they are so. Female animals which are likely to give birth during the transport must be excluded. Consignments of animals must be received and unpacked without avoidable delay. After inspection, the animals must be transferred to clean cages or pens and be supplied with feed and water as appropriate.
REFERENCES