Trouble shooting in colonies of experimental animals

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Introduction

It is widely accepted that the monitoring of the health and welfare of laboratory animals is very important. Indeed a great deal of time is spent worldwide carrying out the necessary techniques (*Beynen* 1991, *Boot* 1988, *Needham* 1979, *Small* 1986). But however carefully programmes are set up there will be occasions when an unpredictable event occurs. This may involve stock in a breeding facility or an experimental colony. The evidence of the apparent problem may be noticed by signs of overt disease or by the experimental data varying from the expected.

In either of these situations there is a need for investigative work to be performed and this is often called trouble shooting.

The trouble shooter (the investigator) is a person who should ideally have an all round approach to the subject of laboratory animal health and who does not necessarily need to be an expert in a particular discipline. They must be able to see the problems in a detached manner so that they are able to consider all likely contributary factors and avoid the potential problem of only identifying some causes of the problem. The scientific background of the trouble shooter does not need to be veterinary medicine but the investigator must, if not a veterinarian, have access to one during the investigation. Also they will need the support of a laboratory.

In this paper the author will describe how to approach the solving of problems in an experimental colony by suggesting routes of investigation which can be adapted to suit individual circumstances. Techniques will concentrate on rodents and lagomorphs but other animals will be included where appropiate.

Principles of trouble shooting

It is important to consider why it is necessary to use trouble shooting in experimental colonies. Most experimenters control their studies with care. Normally the procedures have been checked before and the outcome of the experiment is predictable. Sometimes entirely novel methods will be used but even so they are often based on well tried principles. But experiments still fail even with all the precautions taken.

The size and financial well being of the experimental facility does not confer immunity against failure. Indeed many large companies have problems as well as small university departments. The identification of problems common to all facilities is the basis of trouble shooting.

When problems arise they may be very difficult to detect, so that a great help in problem solving is to encourage an awareness amongst staff of potential problems. For example where an experiment is known to involve immunosuppression staff should be told that it may be possible for clinical disease to be seen during the study. By spotting the situation early the chance of detecting the cause improves.

All workers should be made to understand the appearance of the normal animal to be used for their work. Careful observation by competent staff soon leads to an appreciation of the appearance of normal stock. Once this knowledge is gained the sick animal will be spotted quickly. Physiological parameters are also important such as white and red blood cell numbers. Histopathology also plays a very important role in trouble shooting.

It is a fact that many research workers are not familiar with their animals and are

therefore reliant on their animal caretakers for detailed knowledge of the normal animal.

When a trouble shooter is notified of a problem it is important that the person is not directly involved with the work. This is to ensure that an open approach is made and that no preconceived ideas detract from the investigations. Much of the problem solving may not involve contact with the animals on the experiment but will be spent interviewing staff, engineers and caretakers for example. Time may be spent initiating laboratory investigations and interpreting the subsequent reports. Data from the study will have to be checked and finally a conclusion drawn from all of the information generated. There are many areas to be checked and in the following sections an approach to trouble shooting will be given.

Problem solving

Problems vary in size, some are small and easy to solve whereas others can be very involved and take a long time to rectify. But despite these apparent differences it is possible to adopt a similar approach to trouble shooting.

The investigator must use all the skills available and rely on their experience. It is important for the person to understand the complexities of engineering plant rooms, the production of animals in breeding colonies, animal husbandry methods, animal diseases and many other areas.

The authors approach is to use a check list where the various areas are listed and comments are made. This check list will form the basis of the following sections.

Approaching the problem

When notified of a problem the person must first familiarise themselves with the projects being carried out. The simplest method is to interview key workers. It is necessay to determine the object of the study and to examine the protocols for the work.

Care taken at this stage will save time later. The investigator must look critically at the problem and, when given the details must consider whether for example the experiment is being conducted in a way that should be expected to be problem free.

For example rats were used in a study of their lung permeability (*Jones et al.* 1982). High quality animals (bred in a barrier maintained facility) were used and after issue from the unit were housed in conventional rooms for some time. It was found that the data obtained was not as expected. In this experiment the problem was easily solved by using animals soon after issue from the breeding unit before they became infected by potentially pathogenic bacteria (*Royston et al.* 1983). In this case the experimental design was at fault, the investigation did not take long and it was easy to correct the problem.

But if the answer is not so simple then more detailed steps must be taken.

All areas of the facility must be considered as being implicated. Therefore investigation must include the following major areas; examination of the animal husbandry, source of the animal, environment, experimental design and the experimental data. There are other sections to consider but most are involved with these major areas.

Before discussing further the various investigations some consideration must be given to the conduct of a trouble shooting investigation. It is important to remember that as a trouble shooter the investigator is employed to help the facility. It is not their function to apportion blame for any failures of routines found. It is most important that full documentation be kept as the trouble shooter may have to sometime later return to an old investigation if a query is raised. Confidentiality must be strictly observed and permission should be sought before any documents are copied and removed from site. Also the author has found it of great advantage to have a person to act as a liaison between him and the rest of the facility so there is a central contact. The trouble shooter can

also supply this person with duplicate documents.

Facility

The first area to investigate when solving problems is the facility in which the animals are held. This necessiates a visit to the unit and a full inspection. The size of the facility can vary greatly. It may be very small such as can be found in Universities where only a single room may be the entire animal facility or a suite of rooms as found in toxicology units.

It is important to establish basic details about the unit. Is it a conventional unit, a minimal disease unit or full barrier unit? If a suite of rooms is involved are the problems confined to one room in the suite or all the rooms? Do the rooms connect with each other by ventilation shafts, corridors or perhaps by vacuum cleaning tubes? Staff may mix freely between rooms.

The answers to these questions very quickly builds up a picture of the containment of the problem and obviously may start to provide information about how the problem arose. This is particularly important when several rooms are involved as there is almost certainly a common factor which needs to be identified.

The investigator should have access to plans of the facility. It is not uncommon for persons working in a building to have no idea of where air supply is taken from, air handling equipment is located, the location of any filters or baffles in the air supply and this is available on the plans.

Animals

There is a tendency to blame the animals when the results are erroneous. Using biological models, however tightly the experiments are controlled, the data obtained is always liable to be subject to some variation in the results from one study to another. The trouble shooter must be able to check the data from the studies in case the variation in results falls within the permitted range of biological variation. In other words a perceived problem does not exist in reality when investigated. This is a fairly common finding in experiments involving deliberate infection of animals. The variation in data seems to be too great but when statistical analysis is applied the data falls within acceptable limits.

But assuming the problem is real then the animals must be investigated. Their source must be determined, have they been home bred at the facility or were they purchased? Irrespective of where they were produced it is important to know whether the breeding unit was a full barrier facility, part barrier or non barrier unit. Animals produced behind a full barrier may still be carrying potentially pathogenic microorganisms but their standard is not likely to vary over a long period of time. Whereas those produced in a non barrier unit, will probably have a constantly changing microflora over a period of time. This can result in confusion when this type of animal is used in repeat studies.

All animals used in research should be accompanied by health data relating to their production unit. Ideally every batch supplied should have a health certificate. It is necessary for the health reports to be filed so that they can be inspected. When studying a report it is important to note several important points. These include the age of the data, the number of animals examined, the sex and age of the animals examined and which microorganisms have been sought and by which methodology. Recommendations have been prepared (*Kraft et al.* 1992) for the examination of European breeding units which give a detailed breakdown of the common species. It is unfortunately often seen that health data is old and this can mislead the investigator. Male animals having a greater respiratory minute volume (*Guyton* 1947) are better for the checking of potential respiratory problems. Therefore results from female animals may be of less value (*Needham* 1979). The statistics of animal sampling are well described (*Rapp* 1992). Even so

health reports, particularly those from in-house units, show numbers of animals examined that fall short of a suitable number. Reports do not always show the type of production unit with relation to its barrier state. These facts must be sort by the trouble shooter.

An even greater problem is determining from a report what the laboratory examinations involved. Many health reports are negative reports, that is they only show which organisms were not found. Therefore the investigator is left with the problem of not knowing whether the absence of a particular organism, which he feels could be implicated in the problem, is because it was not present in the animals or it was not sought by the laboratory.

A problem arises as to the suitability of particular animals for the purpose they have been purchased. This can be illustrated by reference to *Bacillus piliformis.* This bacterium, currently the subject of much debate, occassionally causes overt disease in young rats. Many experiments are successfully completed every year in rats that have been obtained from colonies containing the organism. But animals supplied from the same infected colonies are totally unsuitable for studies involving the administration of immunosupressant compounds as there is a real danger that clinical disease will appear and animals may die. Furthermore the entire facility may be infected. It must also be considered unwise to use animals for respiratory studies if they are known to be carrying a potential pathogen such as *Pasteurella pneumotropica* or *Mycoplasma pulmonis.* A recent paper set out in detail the disruption to experiments in guinea pigs caused by parainfluenza virus 1, 2 and 3 (*Porter & Kudlaez* 1992).

Having obtained as much information as possible about the animals in use it is often necessary to submit a sample for laboratory investigation. This may involve taking animals from the supplier if the original health reports are considered insufficient. Thought should be given to the statistics of sampling but in many instances a statistical sample is not possible. In these cases the investigator should obtain the best sample possible. When already sick animals are in the colony it is important that at the stage of sampling some apparently normal animals are taken for examination.

The trouble shooter may need to consider the use of sentinel animals to help with the investigation (*Needham* 1979a). These animals, with their known microbial flora, can be used very successfully in an investigation where for example a study with small numbers of animals is being investigated. Also they can be used to check other areas of the facility where animals are not housed but where the areas may be implicated such as a common user procedure room or the cage wash room.

The tests carried out on stock can vary greatly. It may involve only the need to take a blood sample or a culture swab. But it can also involve a full necropsy with specimens being collected for a wide variety of laboratory investigations. The necropsy is best conducted by an expert who is familiar with the procedures for performing detailed necropsies. It may be necessary to consult prior to the necropsy with other workers such as pathologists in case any specialised fixatives are required for tissues.

Staff

Staff routines can provide clues as to the source of problems and in any investigation this area should be checked. It is easiest to consider splitting this section into two parts, considering husbandry staff as one section and the staff working on the study as the other.

The caretakers who have the responsibility for the daily care of the stock are often first to notice the problem. Therefore the details they can provide are invaluable. They should be asked to describe their working practices and the trouble shooter should pay particular attention to any deviations from

what would be assumed normal for the particular species. It is important to ask them for details of which areas they are allowed to enter as this can reveal a problem of cross infection. In some institutions staff are microbiologically tested and this information should be sought. By careful observation other facts can be gained such as the cleanliness of their clothing is a good guide to the overall cleanliness of the institution. If records of in-house training schemes are available, they should be read.

The study staff should be interviewed and asked about their work. It is sometimes the case that they have made a simple error or are unaware of the problem. If the animal has to be inoculated with any substance it is important to ask whether the substance has been checked for sterility. Often, in the authors experience, this is thought not to be necessary. But the intraperitoneal injection of a contaminated substance can lead to clinical infection which has nothing to do with the animals or the facility. This type of problem is easily avoided. Details of any compounds used must be sought with particular attention to their immunosuppressant properties. If there are any then latent infection in the stock may well become an overt problem.

A review of any experimental data already obtained may yield further help in tracing the problem. For example, weight loss seen in control and treated groups may indicate a viral infection. The viraemia is not always detectable as it causes only slight clinical signs. Weight loss may indicate infection. Therefore serological tests can be performed.

The investigation of problems in animal facilities is not always limited to the buildings or the livestock. It is necessary sometimes to undertake detailed studies to investigate a problem in staff which may be related to the animals they work with (*Kennedy et al.* in press).

Environment

This section is one in which many different areas can be considered. The environment is often relegated to a quick thought at the end of an investigation. This is wrong.

Often it is assumed that problems with laboratory animals must be due to the microbiological cause. But failures can arise due to other factors. The environment of animals is very important and should not be neglected. Failures of the environment can influence the outcome of research projects.

As so many different areas contribute to the environment they will be considered separately.

Air supply

The quality of the air supply to rooms where experiments are conducted will depend on the air handling system which may be the simple method of open windows or a sophisticated HEPA filtered system. Laboratory examination of animals can reveal deficiencies of both systems. It is widely accepted that lung tissue should be sterile when cultured for bacteria. But the author has found consistently that when the air quality is poor lung tissue becomes infected by bacteria. The animals may be clinically healthy but the presence of the organisms reveals the problem. The secondary investigation will determine why there is the failure in the air supply. It may be due to a too low flow rate of air due to blocked inlet filters or the use of a system too small for the area it is supplying. In other cases the air supplied may be of sufficient volume and quality for the room but due to overstocking the air is overloaded with bacteria.

The maintenance of a correct air supply to isolators is of paramount importance and it is possible to check the efficiency of their filters (*Needham* 1980).

The checking of the air supply is not always easy and may require specialist assistance.

Water

In general the water supplied to facilities in Europe is of good quality and many workers do not consider it necessary to carry out any treatment of the water. But this can lead to problems. The use of header tanks to supply

animal rooms can also supply bacteria, fungi and protozoa to rooms if the tanks are not inspected and checked regularly. It must also be remembered that water can supply chemicals harmful to animals.
During a problem solving exercise water testing must be considered.

Diet

The quality of animal diets varies greatly. It can be purchased unsterilised, pasteurised or sterilised. Frequently diet is used without any checks and unless requested by the user most suppliers do not supply certificates of quality analysis with diet deliveries.
Microorganisms, particularly spore bearing bacteria but also coliforms such as Salmonella species, can be present in diets. Sterilised diet should be free of microorganisms but the author has experience of the recovery of bacteria from irradiated diet samples. It is a reasonably simple task to check diets for microbiological contamination and should be carried out routinely.
The examination of diets for chemical contamination is very complex and must be referred to a specialised laboratory. Diet can be analysed for vitamin concentrations and this should be requested when for example animals are seen to bleed unexpectedly suggesting vitamin K deficiency or guinea pig teeth overgrow in lactating females due possibly to vitamin C deficiency.

Barriers

If the environment is maintained behind barriers their integrity must be considered. This must involve a critical appraisal of their efficiency and maintenance and may need direct observation by the trouble shooter of procedures such as the introduction of food to a full barrier unit.

Apparatus

In this section the author considers the apparatus needed specifically for a study and for the maintenance of the animals.
Common user equipment is always to be regarded with suspicion. Chief of these is the cage washing facility. So often it is not realised that washed cages can be the source of the spread of disease around an animal facility. It is possible to monitor their efficiency (*Needham* 1979a) and this should be done particularly if a parasitic problem is suspected as parasites will survive the improper washing of cages easier than bacteria.
In some facilities animals are moved from their holding rooms to a comming user procedure room. In the room they may be weighed. The author has found that containers used for the weighing are left on the balance and are not always cleaned between batches of animals. This is a very obvious source of cross infection.

Interpretation

During the investigation of a problem involving a laboratory animal facility it is possible to generate a great deal of data. At the end of the exercise it is necessary to examine these and to reach a conclusion which will be of help to the institution concerned.
At this stage it is easy to rush the process and to give the institution false conclusions. Perhaps the greatest area of potential risk in this regard is the interpretation of any laboratory reports that have been generated in response to specific investigations. It is crucial that the trouble shooter considers all aspects of the report but most important is the methodology used. Lack of care in considering methodology can cause further problems. This is easily illustrated by reference to serological testing. There are several methodologies available for the determination of virus infections in animals using their serum. Modern techniques use ELISA or immunofluorescence. Both of these are widely available but, some laboratories still use haemagglutination inhibition methods which are less sensitive. Therefore the investigator must take this into account if results have been generated by this older method. Also it is important to remember that all techniques can give false positives and there is some evidence that the very old animal is more prone to this hazard than young

animals. This is important because trouble shooting seems to be necessary more often with long term studies than short studies. Histopathology results can be an invaluable backup to serological diagnosis although even this method can lead to false conclusions. It is possible histopathologically to diagnose many conditions that would not be noticed by other methods. A decision must be made as to whether the findings are implicated in the problem or are just an interesting incidental finding.

Summary

In this paper a guide is offered for the investigation of problems in experimental animal colonies. It can not be an exhaustive list of all the potential areas to check. The process has to evolve as the checks are made and the trouble shooter has to adapt to each individual situation. But it is hoped that by discussing the important sections that are likely to involve most problem solving exercises those working in this field will feel more able to carry out their task.

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