Module 19

Welfare of Animals used in Education, Research and Testing

Lecture Notes

Slide 1:

This lecture was first developed for World Animal Protection by Dr Greg Dixon (University of Bristol) in 2003. It was revised by World Animal Protection scientific advisors in 2012 using updates provided by Dr Caroline Hewson.

Slide 2:

This use of animals in education, research and testing is very contentious in many societies, and we only have one lecture to consider the topic as a whole. We will start by reviewing the ethical arguments (they are outlined in more detail in Module 12), and then we will outline some of the wider psychological and institutional reasons why we use animals for research. Our goal here is to help you to recognise the biases at different levels that can affect both the opponents and the proponents of this animal usage.

We will then focus the rest of the lecture on the welfare concerns relating to each type of usage, and the non-animal alternatives that exist.

As with all the other areas covered in this course, the welfare of animals used in research is a large and growing scientific discipline and laboratory animal medicine is a specialisation within veterinary medicine. However, if you should take a research or teaching path in your career, today's lecture provides you with a very broad overview of the animal welfare issues involved, and some solutions.

Slide 3:

Animals have been used for research and educational purposes in many countries for many centuries. However, the amount of uses has burgeoned since the nineteenth century. Today, there may be over 100 million animals used every year in laboratory experiments worldwide, and rats and mice are the most commonly-used species. Some animals are also used in education at schools and universities as well as for military and space research; for example, by the Department of Defence.

These usages of animals have caused growing ethical concern for decades. This concern started primarily in European countries, along with North America, Australia and New Zealand.

However, it is now global, as you can see in the examples on the slide – Brazil, African countries, China, Turkey and Iran.

In addition, the World Organisation for Animal Health (OIE), which represents 178 members, has specific guidelines on the treatment and handling of animals used in research. You can find these online in its Terrestrial Animal Health Code. They represent minimum standards for the care of laboratory animals and are not legally binding, but could provide a basis for animal protection legislation in countries that do not yet have legal protection for the animals concerned.

The use of animals for research, testing and education creates moral unease for many people because of concerns that:

- animals are often subjected to invasive procedures that cause immense harm and suffering
- the animals' housing and care does not benefit them, and little or no effort is made to mimic their natural environment and allow for natural behaviours such as nest-building in mice, which often causes stress to the animals
- the benefits to people of doing research on animals is limited.

While animal welfare science and the use of ethics committees are enabling us to address the first two points, the third is less well received by many of the public –especially in the case of research into human diseases – and by most researchers and other stakeholders.

So, before we consider the welfare issues in the procedures and husbandry, we will look briefly at the history of this animal use, and why its alleged benefits are controversial.

Slide 4:

The history of animal use arose from Western ideas about science; however, note that other cultures may have approached the acquisition of biological knowledge using different assumptions and more humane frameworks.

Briefly, the very early Christian church forbade the use of human bodies for scholarly study, so animals were used instead, for many centuries.

In the nineteenth century, the physiologist Claude Bernard (who developed the concept of homeostasis) emphasised the necessity of using animals in the laboratory in order to advance human medicine. He is quoted as urging researchers not to listen to animals' cries or to regard any haemorrhage as they worked on important questions of human health. His intention, as with researchers generally, was not to deliberately harm animals but rather to solve serious problems of human health.

The undoubtedly great discoveries that were made, and the thinking of scientists such as Bernard, set the stage for the assumption that research on animals is essential for human medical advances. In those days, such use of animals was the best available tool. It was only during the twentieth century that ancillary developments in pain relief, enriched animal housing, statistics, mathematical modelling, tissue culture and computers made alternatives available.

Meanwhile, the original ‘path’ become entrenched as the primary working method, i.e. that it was acceptable and a necessary evil to use animals for research and education.

Slide 5:

In other academic disciplines such as economics and psychology, scholars have examined how such paths become established.

Economists propose the concept of ‘lock-in’ whereby, if a chosen path of action provides increasing returns, this gives rise to inflexible behaviours and strongly-held assumptions about the path chosen, even though a different path might be more beneficial in the long term.

Note that, at the outset of new endeavours, no other path for progress may present itself. Also, it is impossible to know that the first path chosen will be more beneficial than any other. If the chosen path provides increasing benefits, it is difficult for people to change it and trust that any other path could be better.

The use of laboratory animals for medical research seems to fit the lock-in model. In the case of drug development, there is a very big risk and initial investment in the development of each potentially useful molecule by pharmaceutical companies – only one in ten potential drugs proves to be successful, and each drug takes around seven years to establish before any return can be gained by the company marketing it.

However, for drugs that are shown to have clinical benefit, there are increasing returns, and companies can recoup the considerable investment they made at the outset. In addition, if a drug has been proven to be useful clinically there will be a huge non-financial benefit to the users, with indirect cost savings from their increased health.

The use of animals is part of achieving all these benefits, so that path is therefore set.

Continuing our extrapolation from the economics literature, ‘lock-in’ is associated with collective inertia and reluctance to change the existing path. This inertia exists for several reasons:

- the path now has a large interconnected infrastructure. In the case of the use of laboratory animals, that infrastructure includes companies breeding the animals, the various laboratories, the cosmetics, pharmaceutical and chemical companies which are developing the products, all the personnel concerned, the academic departments, funding agencies, regulatory bodies, professional academic journals, associations and conferences
- to change to other methods involves a cost, for example, what to do with animal housing if animals were not used, and so on.

Slide 6:

Numerous psychological factors may also contribute to inertia. Note that these factors do not simply apply to those who may be accustomed to using animals for research. Neither have they all been studied in that context; but they are common psychological traits that human beings may develop in various contexts.

The factors may apply as much in those who are opposed to the use of animals for research as in those who believe that it is necessary. They may apply in this lecture too, as there is no time to cover the entire field in detail so, on this slide for example, we are only looking at some types of bias – which is itself a bias, however unintended!

Having acknowledged that, we now look at examples of psychological factors that may contribute to the use of animals in research being entrenched.

The first factor is bias. There are many more, including:

- a tendency not to use information that is removed from or very different from one's local experience – some researchers or educators who use animals may tend to resist information about alternatives in their case
- publishing bias is a problem across academic research, in all scientific disciplines: it is the tendency of editors not to accept for publication those papers that fail to find a difference between two treatments
- the tendency among editors, researchers and journal readership to assume that because a difference is statistically significant, it has any biological/relevant meaning in reality. Overall there tend to be more papers published about research using animals that found 'statistically significant differences' than there are papers about research that used animals and found no differences, as this adds little to the field of research. This is also a concern, as it means that scientists cannot share their information and could mean the research is repeated and more animals suffer unnecessarily. This reinforces the impression that using laboratory animals is essential for medical developments
- confirmatory bias: this is the psychological tendency to resist evidence that contradicts what we believe to be true, and instead to persevere with our own belief. An example is resistance to the opinion given in critical reviews of clinical research, that research on animals has provided little or no benefit to people, particularly in cancer research. Note that confirmatory bias may be found in opponents of research too, e.g. they may be unable to accept evidence that laboratory animals can have very good lives, in each of the three aspects of welfare.
- related to confirmatory bias is the idea of 'internal psychological appeal' where aspects of the issue of concern are described in terms of desirable features. In the case of using animals for research, such labels are that the studies are 'objective', 'controlled', 'quantitative' and 'scientific'

- a further point is that, in some countries, the use of animals in research has given rise to some extremist animal liberation groups. They are a minority, and some of their members can be angry, disaffected and violent. But groups receive a lot of media coverage, and because of this those using animals for research may associate any criticism of their work with those violent and vocal minorities, and dismiss it, rather than give it objective consideration.

Slide 7:

Cognitive dissonance applies in laboratory animal research too. Module 30 on human–animal interaction explains *cognitive dissonance theory*, which states that if we hold conflicting or incompatible views, this causes us to experience unpleasant emotions, and we will change our behaviour or our attitudes in order to overcome this. This may be reflected in the language that we use. For example:

- when laboratory animals are killed for research purposes, the common term that is used in the literature is that the animals were 'sacrificed' – this terminology connotes value, ritual and respect. (Similarly, when owners or vets discuss euthanasia of their animals, euphemisms may be used such as 'put them to sleep' or 'put them down' (in the UK), or 'euthanise them' but not 'kill them'
- however, when the data from research animals are reported, the animals typically do not have names, only numbers. That impersonality creates distance from the animal as a sentient being with personality, who might have been harmed by the study.

A final point is that people working with laboratory animals form an emotional bond with them, which is often affectionate. For example:

- laboratory personnel may single out certain animals so that they are not used in experiments or scheduled for euthanasia. In some cases, staff members may adopt an animal
- conversely, to avoid the discomfort that a bond creates, some technicians and researchers may avoid visiting or handling the animals
- either approach may affect the animal's experience of his or her life, including physical functioning and behaviour. Some research indicates very clearly that this may skew the results of studies, and may even confound them such that an apparent treatment effect may simply be a response to the handlers or the stress inflicted by the handling or treatment procedures.

These points illustrate the complexity of psychology and other factors that surround the use of animals in research.

Slide 8:

Next, we shall review the ethics of using animals in research, education and testing. Module 12 looks at the arguments in more detail. Briefly, we can say that, as well as the various historical and psychological reasons for using animals in this way, the prevailing ethic is largely utilitarian: the usage is justified if the benefits to people (and to other animals) outweigh the costs paid by the animals in the project concerned.

Since the 1950s, the concept of the three 'Rs' (referred to as '3Rs') has been used to help minimise that cost. That is:

- **replace** sentient animals altogether where possible. However, if animals must be used:
- **reduce** the number needed to the minimum in order to measure the effect of interest, and
- **refine** the methods so as to minimise any harm caused to those animals.

These three principles represented a major advance in the more humane treatment of animals in research and education, and we will now look at the 3Rs in more detail.

Slide 9:

The 3Rs were defined by two UK scientists, William Russell and Rex Burch, in 1959, in a book about humane animal experimentation called *The principles of humane experimental technique*.

Since then, the 3Rs have been embodied in the legislation of some countries and supranational bodies, such as the European Union (EU).

Also, the OIE cites them throughout the chapter of its *Terrestrial Animal Health Code* on the use of animals in research education. However, note that the 3Rs apply equally to aquatic animals and all sentient species.

The 3Rs are centred on using alternatives wherever possible, i.e. "any method, technique, proposal or approach" that avoids or minimises the use of animals.

The slide shows the OIE guidelines on the 3Rs. Their exact wording is as follows:

1. "Replacement refers to the use of methods utilizing cells, tissues or organs of animals (relative replacement), as well as those that do not require the use of animals to achieve the scientific aims (absolute replacement);
2. Reduction refers to the use of methods that enable researchers to obtain comparable levels of information from fewer animals or to obtain more information from the same number of animals;
3. Refinement refers to the use of methods that prevent, alleviate or minimise pain, suffering, distress or lasting harm and/or enhance welfare for the animals used. Refinement includes the appropriate selection of relevant species with a lesser degree of structural and functional complexity in their nervous systems and a lesser apparent capacity for experiences that derive from this complexity."

Opportunities for refinement should be considered and implemented throughout the lifetime of the animal and include, for example, housing and transportation as well as procedures and euthanasia.

Later in the talk, we will look at real examples of how the 3Rs are applied.

Slide 10:

Returning to our discussion of ethics, however, note that some scholars do not accept the utilitarian view of research – and therefore the value of the 3Rs – because both are an acceptance that research can benefit people, and that animals do not have intrinsic value (rights). Module 12 examines this in more detail.

A further difficulty is how exactly we weigh up the burdens carried by the experimental animals (e.g. restricted housing, fear, pain) against the potential benefits to people or to other animals.

For example:

- many new commercial products (household cleaners, cosmetics and drugs) are not essential for human or animal health, as they are no better than the ones that are already on the market. However, the licensing of each one involves toxicity and pharmacokinetic studies on laboratory animals. Moreover, although countries are becoming more accepting of data generated in other jurisdictions, some countries may only license the product if the safety and efficacy data have been generated locally. This can mean unnecessary replication of animal use
- some of the conditions being researched are not life-threatening, e.g. baldness in men
- more importantly, data derived from the laboratory animal may not predict the action of a drug or vaccine in people. One example of this is the polio vaccine, which is discussed on the next slide.

Slide 11:

Poliomyelitis is an infectious viral disease that causes paralysis and muscle wastage in children; the paralysis may cause death. Humans are infected orally, by contaminated food and water. Vaccination has eliminated the disease in 99 per cent of the world's population.

Research to develop a vaccine took place in the first half of the twentieth century, when tissue culture and other modern techniques were not developed.

The virus can only survive in living primate tissue; therefore, it was grown in monkey kidney tissue, and vaccine safety and efficacy were tested on monkeys. This is thought to have used one million monkeys (mostly rhesus macaques) and may have reduced the Indian macaque population by more than 90 per cent at the height of the trade.

Researchers infected monkeys through the nose as this was thought to be a likely route for human infection, despite evidence from human clinical studies that large amounts of the virus were found in the intestines. However, it turned out that humans require oral infection, and effective vaccines only resulted after the virus was grown in human tissue. A successful oral vaccine was produced in the early 1950s.

In retrospect it was clear that the research on macaques did not bring many benefits. More notable is that the work with them persisted in part because the data from observational studies in human patients was ignored at first, perhaps because the observations were considered less scientific than 'controlled' laboratory-based animal work.

This case study illustrates elements of 'lock-in' and welfare issues in the use of wild, non-human primates in research.

Slide 12:

In light of all the factors around the use of animals in research, ethicists at the Danish Veterinary School in Copenhagen have suggested that animals should only be used if the following three conditions are met:

1. The research question must be of vital importance (the definition of 'vital' would be controversial for many chemical, cosmetic and pharmaceutical companies and their investors – which include pension funds – as companies which develop 'copycat' products to increase their profits in a free market create a lot of wealth and employment).
2. There is no other way to study the issue except by using animals.
3. The animals do not have to suffer more than the experiment requires; for example, they should have enriched housing unless that would confound the results.

You can see from this very brief introduction that there are many unresolved issues surrounding the utility and acceptability of using animals for research, testing and education. In the meantime, usage continues and, as veterinarians, you may be employed in a research laboratory or do research on animals yourself. In clinical practice, and in your daily life, you will use products that have been developed using research animals. As students, you may use animals too.

In light of this reality, we shall focus the rest of this lecture mainly on:

- the effect of procedures on the welfare of laboratory animals used in research, education and testing
- the effect of husbandry conditions on their welfare.

Slide 13:

We start with the welfare of animals used in teaching.

Approximately 2–3 per cent of laboratory animals are used in schools and in undergraduate teaching.

Traditionally, this usage has been seen as the only way to teach certain skills (e.g. surgery for doctors and for vets; rectal palpation of cattle for pregnancy diagnosis) or convey certain types of knowledge (e.g. to demonstrate the effect of drugs on heart contractility in a live animal; to teach anatomy through dissection).

For example, in some veterinary programmes, and in other university-level training, e.g. in psychology, animals repeatedly undergo invasive surgeries, from which they are allowed to recover and which they may not need, for the purposes of teaching students surgical techniques, anaesthetic monitoring, etc. However, there is growing consensus that the uncritical usage of animals for education creates an unfeeling and utilitarian attitude to living beings, which is especially paradoxical and potentially problematic in veterinary training.

Also, research increasingly indicates that the usage is not necessary for the purpose, in most instances, and that other methods that do not harm animals are just as effective.

Moreover, in the case of biology classes at secondary schools, or even in undergraduate classes, it is hard to know how many students will go on to use the knowledge gained from the class with real animals in their careers, so the argument that the usage will have future benefits to society cannot be substantiated.

There is a lack of data on the scale of usage or on the effectiveness of animal usage on learning outcomes, when compared with non-animal alternatives. For example:

- animals who are killed prior to the class, e.g. for dissection, are not included in most countries' calculations
- at school-level, in particular, there are concerns that species such as frogs and fish, which are used in dissection, may not be killed humanely
- a literature review of controlled studies comparing methods that require the terminal use of animals with alternative teaching methods (Patronek & Rauch, 2007) only found 17 controlled studies between 1996 and 2004. All those studies indicated that non-animal alternative methods of teaching were not significantly superior to the traditional ones, but were equally good.

We now move on to the welfare concerns. Animals used for education may be kept in barren housing and may not be given adequate pain relief following surgeries. Module 9 provides you with information about animal welfare assessment, and individual modules about different domestic species review the common welfare problems with housing.

Slide 14:

As with animals used for research, usage of animals in the veterinary curriculum has probably been subject to some of the inertia and psychological complications that we described in the introduction to this lecture. This has historically created problems for students who have to train without harming animals. Those students might be said to be 'conscientious objectors'. Practical points in such disputes included:

- in a class where the teacher is unaware of alternatives, and where the university has a very limited budget for teaching resources, it is difficult to accommodate a student who has ethical objections to the training method
- the teacher has the obligation to ensure students are trained, and may not be equipped to convey the information required using new techniques at short notice
- in turn, the student has a requirement to acquire the skills needed to fulfil the public's trust in his or her professional competence.

Keeping these points in mind, and noting the risks of 'lock-in', when students raise concerns about using animals in their veterinary education, the situations can become a constructive opportunity for raising standards in the long run.

This brings us to applying the 3Rs in veterinary education.

First, many universities have a dedicated committee with faculty members and sometimes members of the public too, who have to approve any use of animals for teaching, and would typically use the 3Rs as a framework. The teacher would have to demonstrate what usage was necessary and how the 3Rs were being followed.

Second, veterinary educators are increasingly aware of the 3Rs, and there are many innovative alternatives available to help meet the 3Rs.

The slide cites two papers on the subject from the *Journal of Veterinary Medical Education* (2005), and there are various international educational conferences on the subject of alternatives to animal use.

One of the biggest is organised by an educational company called InterNICHE, which produces many innovative educational tools that are alternatives to using live animals.

Examples of each of the 3Rs in veterinary education are listed below. Researchers at the vet school in London have developed a haptic cow to teach abdominal anatomy and rectal palpation. '*Haptikos*' is a Greek word for the sense of touch, and the haptic cow is a computer simulator whereby the user can touch and visualise a cow's internal organs, virtually, in 3D. In initial research, it was used to teach abdominal anatomy to vet students in groups of five to seven students, over just 20 minutes. The students enjoyed it, and felt they learned exactly where structures were, despite the short time available. The haptic cow is now used at several veterinary schools, and it has been validated as a way to teach the clinical skill of rectal palpation as well.

Other simulators have been developed and are being developed for other species.

A second example of absolute replacement in veterinary education is a multimedia tool to teach equine obstetrics.

This is a DVD called *Foal in Mare*, developed at the Ghent Veterinary School, Belgium. It uses auditory, textual and visual representations to provide a 3D film that enables you to see inside the mare through translucent organs.

Preliminary research indicates that students who use the DVD prior to the lectures on obstetrics demonstrated significantly more confidence in their understanding, based on written tests, and needed less mental effort to answer the questions.

Slide 15:

Here we move on to (relative) replacement in veterinary education. A widespread example is the use of preserved body parts to teach anatomy. New plastination techniques to preserve or create models of the structures mean that they can be reused repeatedly rather than needing new specimens all the time.

Note that the animals should be sourced ethically, and humanely killed.

The use of stray animals would not normally be acceptable here, although – depending on the local context and carefully considered ethical position – the use of animals who had been euthanised at shelters might be preferable to animals that were kept in barren conditions at the university for the purpose.

The next 'R' is reduction in numbers of live animals used. Examples include:

- 'educational memorial programmes': when an animal dies, the owner donates the body to the vet school and students can practise intubation and surgery on the body
- newer preservatives that keep dead tissue flexible so that ethically sourced cadavers can be used for surgical practice, rather than purpose-bred live animals
- cows who are going to be slaughtered at the abattoir have been used to teach rectal palpation. That has proved useful educationally, and it avoids the need to keep cows just for that teaching purpose. However, as you will know from the modules on livestock welfare, and the modules on transport and slaughter, it seems likely that the animals concerned are already at a high risk of being distressed while at the abattoir, and being used as teaching animals may not be in their interests
- extramural studies are a very important form of reduction, whereby vet students 'see practice' and are mentored by an experienced vet. Attending client animals under supervision and with the client's consent has long been a way to teach students surgical and other skills. It removes the need for students to carry out survival surgeries on animals kept at the university.

Slide 16:

The third 'R', refinement, applies to all live animals on whom students practice their skills and otherwise gain knowledge.

As noted in the OIE's guidelines, refinement refers to every aspect of the animal's life, so it would also include the animal's housing and handling, diet, transport and euthanasia. (This resource includes modules on each of these topics.)

Examples of refinement during the teaching session are: the use of pain monitoring and analgesia, gentle handling, and restricted number of uses per teaching session, so that the animal's welfare is always paramount.

Note that in many veterinary schools, the use of animals for recovery surgeries or for surgeries that the animal does not need is prohibited. However, where it is still practiced, the principles above help refine the usage.

Slide 17:

We now move on to the welfare of animals used in research and testing. This accounts for the vast majority of laboratory animal usage, and it encompasses many types.

Broadly, medical (and veterinary) research on animals is centred around three models of research.

- Exploratory models: these generate information, the application of which is not immediate. If the information should have application in future, this may not yet be obvious. This type of research raises the concern of utilitarianism as an ethic of animal use, since it is impossible to calculate if exploratory research on animals will ultimately have any benefit to humans or other animals. However, the compromise ethic that we noted earlier may allow some of this research if it is vital for human beings.
- Explanatory models: these are designed to discover mechanisms, such as the mechanisms of cancer development, aging, drug activity, anxiety, etc. This is an area where genetically modified animals may be used, e.g. mice with a gene that predisposes them to develop a specific cancer.
- Predictive models: these are practical, and allow us to solve problems and make practical decisions concerning the efficacy, potency and safety of products such as drugs, vaccines, household chemicals and even food, e.g. mice are used to test for toxic substances that shellfish may produce.

We shall now look at some examples of the welfare challenges and the use of the 3Rs in some of these types of research.

Slide 18:

In veterinary practice, much of our work is preventative and includes vaccination. The production of these vaccines creates various animal welfare concerns because each batch of vaccines has to be tested for each of the following:

- safety – the vaccine should not cause adverse effects, e.g. a modified live vaccine should not revert to virulence; with a killed vaccine, the adjuvant should not cause a local reaction
- purity – no additional substances that might cause adverse effects, e.g. few or no residual proteins from the cells in which the vaccine virus was grown, and which might cause an anaphylactic reaction in your patient
- potency – enough of the antigen in question, sufficient to create an immune response
- efficacy – adequate protective immune response.

If the batch is not tested in all these ways, the risk is that the animals you vaccinate in practice either will not develop a satisfactory immune response, or may suffer adverse effects.

Some of that testing can be achieved using in vitro testing, not live animals. However, some live animals must be used and may suffer a great deal.

Researchers, regulatory authorities and the vaccine companies are very aware of this and they all met in 2010 to review how to apply the 3Rs more widely in veterinary vaccine production.

Slide 19:

The example we will look at is potency testing of veterinary vaccines. The procedure traditionally goes like this:

"Serial dilutions of the vaccine are administered to several groups of animals. A group of control animals is included that do not receive the vaccine. All animals are then challenged with a dose of live pathogens sufficient to cause clinical disease in unprotected animals in order to determine the quantity of vaccine that provides protection. Inadequately protected and unprotected control animals typically develop clinical disease or die" (Stokes et al., 2011, p.6).

The welfare concern here is the choice of end-point. A humane end-point is the moment in the study when an experimental animal's suffering is terminated by, e.g. analgesia, euthanasia, removing from the study.

With vaccine potency tests, the end-points have often *not* been humane, with animals allowed, for example, to have pyrexia and anorexia until they eventually die. Here we should apply the principle of refinement which, you will recall, is about minimising any distress to the animals. We need more research on each disease of interest so as to identify the earliest clinical signs that predict death in protected animals during the challenge test, e.g. it might be once an animal's temperature exceeds a certain figure for a certain minimum time.

Training is needed so that personnel recognise those signs correctly and so can safeguard the welfare of the animals in the challenge test, on the one hand, and the welfare of animals in the field who will be vaccinated using the batch concerned.

The goal would be for personnel to:

- Remove those animals in the challenge test that are about to become mortally ill at the earliest opportunity, so that the animals may either be euthanised or treated and, in either case, may avoid unnecessary suffering. This is essential for the welfare of the laboratory animal.
- Not remove the animals prematurely from the test such that their immunity – and thus the efficacy of the batch of vaccines under test – is not properly assessed. Proving the efficacy of the batch is essential to ensure that animals who will be vaccinated in the field are adequately protected.

A further refinement is that, for some veterinary vaccines such as tetanus, swine erysipelas and clostridial diseases, there are now in vitro tests of potency, so that live animals do not need to be challenged.

However, although some countries have reviewed these tests and accepted them instead of live animal testing, other countries have not considered them.

Reduction of animal use for potency testing includes:

- only using the minimum number of animals per group
- in combination vaccines, carry out the potency test on the combined vaccine, rather than having separate groups of animals to test each component.

Similar issues with the 3Rs are also recognised with fish vaccines.

Slide 20:

We shall now move on to the wider use of animals for toxicology tests.

About 10 per cent of all laboratory animals are used for toxicological and other safety evaluation of household chemicals, veterinary and human pharmaceuticals, cosmetics and some food. Many of these tests are, by convention, animal tests and might include:

- acute toxicity tests (effects of large doses of products) and chronic toxicity tests (effects of smaller doses over long periods)
- eye and skin irritation tests, for example, the Draize test, which involves dripping substances into rabbits' eyes. The picture shows a typical system that was used historically
- the Draize test has been banned in most countries but is still used to test some non-cosmetic chemicals that might come into contact with the human eye
- carcinogenicity tests are done to see if the product will cause cancer

- developmental and reproductive toxicity tests are carried out to identify harmful effects on reproductive systems or foetuses – these are done with human and veterinary drugs
- mutagenicity tests are carried out to assess whether products can cause genetic mutations.

A further safety test may be done for some foods. A particular example is a mouse bioassay that is used to detect toxins that cause paralytic shellfish poisoning (PSP) in people. Shellfish are a big export for some countries and it is important, both economically and for human safety, that they do not poison the consumer.

The traditional assay uses a large number of mice and requires death as an end-point. Canadian researchers have developed a High Performance Liquid Chromatography (HPLC) method that is more sensitive and more reliable than the mouse bioassay that has been accepted and adopted by some international bodies.

This example illustrates elements of inertia, discussed earlier, that can result in the 3Rs not being implemented.

Slide 21:

So far, we have looked at the welfare effects of testing procedures – in veterinary vaccine testing and in a variety of toxicology testing. We have also seen how the 3Rs have been implemented in some of these examples. Those have all been cases of predictive research – using animal data to help us plan and make decisions. Next, we shall see how the 3Rs can be implemented in explanatory research, using the example of Parkinson's disease.

This disease "is a devastating neuropathological condition, characterised by progressive neurodegeneration of dopamine (DA) neurons" (Manciocco et al., 2009) in the brain. It affects approximately 1 per cent of the world's population over the age of 55, and causes them to suffer tremors, weakness and depression. The cause is unknown but involves some interaction between one's genetics and the environment, rather than an infective agent.

Rats and mice have been used as explanatory research models to examine the effect on dopaminergic brain structures of pesticides and other chemicals. Transgenic mice are also used.

However, animal usage has been reduced and improved according to the 3Rs, and could be further improved in several ways. Examples are shown on the slide.

- Replacement: there are now many in vitro studies with cell cultures; invertebrates could also be used to study the molecular genetics, e.g. fruit fly, flatworm. However, it must be noted that direct replacement of vertebrate species with invertebrate species does not necessarily meet the replacement criterion: as our knowledge of sentience in animals continues to increase, many invertebrate animals are being found to be sentient and able to suffer (for example, cephalopods used in experiments are protected by the UK and NZ governments)
- Reduction: here, the main points concern good study design and appropriate statistical methodologies

- Refinement: using transgenic mice containing the human gene associated with the disease may be preferable to creating the disease by painful or invasive procedures, e.g. injecting putative toxins into the brain. Other refinements, as we have seen, include the researcher's knowledge and awareness of animal suffering, and the choice of humane end-points. Also enriched living conditions and other types of good husbandry.

These applications all hold promise. However, note that the 3Rs may conflict. For example:

- When researching Parkinson's disease, the reduction and refinement principles conflict if the person carrying out the experiment is injecting toxins to induce the disease in rodents. In line with refinement, the scientist could use lower doses of toxin so that fewer animals die, and fewer develop motor signs compared to if he or she used a higher dose. However, this means that a larger number of animals might be needed to detect an effect, if one existed
- in the case of using transgenic mice as a type of refinement: not all those mice might demonstrate key features of the disease, so you may need to create many transgenic mice, and you then have to care for and use the unaffected ones – or euthanise them.

Slide 22:

This brings us to the use of *genetically modified* (GM) animals in research – and, potentially, for food, companionship, etc.

This involves the manipulation of genes – either within a species or between species. Genes are either 'knocked in' or 'knocked out'. A 'knock-out' animal has a deleted or damaged gene that no longer works. There is a high failure rate in genetic modification procedures. Most animals fail to take up the desired gene and they are killed: only 1–10 per cent of the offspring will incorporate the desired gene, giving an estimated 90 per cent wastage. Apart from moral disquiet about this approach, there are specific animal welfare implications.

First, as we saw just now, the animals develop the disease of interest.

Second, animals may experience problems during their development or unexpected problems. For example,

- some transgenic farm animals have an accelerated growth rate, creating various pathologies; however, further gene manipulation can control this
- some animals may suffer severe, even lethal, unexpected effects, such as the development of tumours, brain defects, limb and skull deformities, infertility, arthritis, diabetes and other metabolic disorders.

Third, there is a general problem of 'wastage', as we saw earlier.

Slide 23:

Examples of applications of genetically modified animals are shown on this slide.

- The production of therapeutic proteins in the milk of sheep, goats and cattle has been done by introducing genes of human origin into the animals. The technique to produce an animal capable of doing this involves surgery on several donor and recipient animals, although the transgenic sheep, once produced, appear healthy.
- Livestock/agriculture: animals have been genetically modified in attempts to make them grow faster, produce more and leaner meat, more eggs, more milk, or more and better wool. Sometimes the aim is to try and improve their resistance to disease, as is often emphasised with GM crops.
- Transgenic animals as models of diseases such as Parkinson's or cancer.
- Xenotransplantation: this includes the production of transgenic pigs to produce organs which are not rejected by human recipients. These are not human organs, but rather animal organs with a human gene which enable the human immune system to accept the foreign tissue.
- In addition to the general welfare concerns of GM animals, pigs reared for xenotransplantation need a disease-free environment, which can reduce their welfare by, e.g. denying them suitable rooting substrates because of the risk of introducing disease.
- To obtain 'disease-free' piglets for organs for transplants, the animals are delivered by Caesarean section, placed in isolators, and then reared in sterile environments; the offspring are also subjected to repeated procedures where blood and tissue are taken.

Arguably, there are still many 'unknowns' in our use of genetic biotechnology, and while more research is being gathered on these issues, there is good reason to opt for a precautionary practice that gives the animal the benefit of the doubt.

Slide 24:

We have now looked at a selection of examples of welfare issues arising from the procedures undergone by research animals, and we have seen how the 3Rs may be applied.

However, anecdotal evidence suggests that few journals require explicit discussion of the 3Rs. Some journals provide guidelines about them, but they do not seem to require discussion of their implementation within the text for a paper to be considered for publication.

Some research has been done on that point. For example, animal welfare scientists have examined reports published in peer-reviewed journals between 2003 and 2004 describing experiments employing animal models to study the neurodegenerative disorder Huntington's disease. They found 51 references to studies where animals were expected to develop motor deficits so severe that they would have difficulty eating and drinking normally. However, only three of those papers referred to making housing adaptations to facilitate food and water intake. In addition, experiments including end-stages of the disease were reported in 14 papers yet, of these, only six referred to the euthanasia of moribund animals.

Many funding bodies and university ethics committees, or national licensing bodies, require clarification of the 3Rs in the applications to carry out research. However, the authors of the review of the Huntingdon's disease papers concluded that many researchers may not be applying the 3Rs.

Interestingly, the reduction principle can be applied effectively in practice by involving veterinary client animals in more types of research, e.g. providing samples, so as to reduce the need to keep laboratory dogs and cats and induce diseases in them.

Also, there is the possibility for some translational research, i.e. clinical research on client-owned animals who have diseases of interest in human medicine. An example of such a disease is canine spontaneous osteoarthritis, which could serve as a model that would more closely mimic the condition in humans than would a laboratory animal model.

Slide 25:

This slides sums up what we have covered so far in this lecture.

- We started by reviewing why using animals for research and education is so widespread, and we considered the concept of 'lock-in' and inertia. We also looked at the main ethical points. This introduced the role of the 3Rs in minimising harm to the animals concerned.
- Next, we looked at the welfare of animals used in veterinary education, primarily, and some of the alternatives to that
- We have looked at several different examples of animal use for testing and research. We considered the use of animals in veterinary vaccine testing, and what some alternatives might be. This introduced the concept of humane end-points.
- Next, we reviewed animals used to test the safety of products, including shellfish for human consumption. With the Draize test and the shellfish toxicity assay in mice, we saw that animals were still being used even when non-animal options were available, largely because of logistical barriers and inertia.
- Then we saw how the 3Rs might apply in explanatory research, using the example of studies of the cause of Parkinson's disease.
- That led us into a review of the use of genetically modified animals, and the welfare concerns for them.
- We finished by considering the role of journals in ensuring that researchers thought more about the 3Rs.

We will end today's lecture by looking more widely at the question of refinement as a way to improve the welfare of animals in their housing, etc., and then at how regulations can help to protect laboratory animals.

Slide 26:

Although much of the debate about the use of animals in research and testing concerns the procedures which animals undergo, most animals spend more time in their housing than undergoing procedures. This is true of farm animals too and, as with them, laboratory housing should meet the animals' physical needs: sufficient food, water, space, hygiene, adequate temperature and veterinary care. Most housing conditions do not go beyond that, and are confining and barren. Module 15 on environmental enrichment (EE) discusses the effects of a lack of stimulation in confinement in more detail.

Briefly, animals in those conditions can suffer boredom and stress. Moreover, rats and mice, the most common laboratory species, are social but are often housed in isolation for ease of the researcher, which prevents the animals from expressing natural behaviour such as play, grooming and social interactions.

'Environmental enrichment' is used to prevent these welfare problems. This is the alteration of the environment of captive animals in order to increase their behavioural diversity and so improve their welfare. Typically, it involves making the structure of their environment more complex.

Examples of effective enrichment for rats that studies show them to value include shelters and tunnels, bedding and nesting materials, and group housing. Similar enrichments are also valuable to other rodents, and recent textbooks on the welfare of laboratory rodents provide more details. Note that the OIE guidelines recognise environmental enrichment as being a minimum standard of care.

Enabling laboratory animals such as rodents to perform a full range of behaviour, including exploration, socialisation and play, maximises their opportunities for pleasure, while reducing the likelihood of them suffering stress. The benefits also extend to the validity of the data that they provide to their researcher. There is a growing body of evidence that traditional barren housing and stressful routine handling can affect laboratory animals' behaviour and physiology, to the point where it interferes with the researchers' data.

This is becoming increasingly clear in the case of behavioural research, where rodents are used as models of anxiety and the effect of drugs with potential to treat depression and anxiety in humans.

Being raised in a barren environment by mothers who were not handled much can affect the animals' cognitive processes.

Moreover, being reared in barren cages may not allow the adequate development of vision, which may affect rodents' ability to perform some of those tests – examples of tests that require visual acuity are a swimming test, and a test using a maze.

Studies of standard handling vs. additional playful and gentle handling also support the likelihood that all these factors strongly affect rodents' ability to perform specific behavioural tests that researchers use to assess anxiety, etc. This therefore calls into question the validity of a lot of exploratory, explanatory and predictive research.

To sum up this slide, we can say that minimising distress by providing enrichment is essential for valid data.

Slide 27:

Social aspects of housing are also very important for the welfare of social species – notably rodents and primates – and for the validity of the data they provide in research. In particular:

- pre-weaning social experiences affect the development of social skills and capacity to cope with stressful situations, so compatible and stable groups are crucial
- kinship is important for the compatibility of both rodent and primate groups
- animals react differently in behaviour tests when tested in a group than when tested individually.

Overall, therefore, social factors are important for laboratory animals' welfare and when planning and interpreting research that uses them.

Slide 28:

The feeding regimen can also be a welfare concern in laboratory animals (see Module 19); Module 15 on environmental enrichment will also provide more detail.

Briefly, the feeding regimen may be *ad libitum* feeding or dietary restriction. *Ad libitum* feeding is unlimited access to food. This allows animals to follow their natural pattern of eating in bouts. *Ad libitum* feeding is standard for laboratory rodents because it is a labour-efficient system that can be mechanised. However, the regiment predisposes the animals to obesity.

Obesity is associated with reduced longevity, as well increasing the risk of musculoskeletal disorders and diabetes mellitus. Obesity is deliberately created in some laboratory rodents in order to study the mechanisms involved.

The second type of feeding regimen, dietary restriction, is intended to prevent obesity. The restriction may be in the amount fed – which tends to leave animals feeling hungry – or in the quality of the diet – adding fibre to reduce nutritional content while allowing animals to feel full.

Rodents used for studies of dietary restriction may be housed singly in order to ensure they receive the exact amount of food required for the experiment. However, rats and mice are social animals and housing them singly can be distressing. However, if rats are group-housed but on quantitative dietary restriction they may show high levels of aggression to other rats.

Slide 29:

Historically, there has been poor recognition of pain in laboratory animals. This is partly because it is difficult to recognise pain in different species, as each one shows different behaviours. For example, a UK researcher showed videos of rabbits before and after having been spayed, to 151 viewers (Leach, 2010). The viewers included veterinarians, scientists, lay people and animal health technologists. Approximately half had experience of caring for rabbits. The viewers looked mainly at the rabbits' faces to assess if the animal had abdominal pain. However, the ability to score pain correctly (according to experts) was correlated with observation of the animals' posterior end, not with observations of their faces.

The researcher noted that, in mice, facial expressions have been shown to be a valid sign of their level of pain. The researcher suggested that this may not be true of rabbits, hence many observers did not assess the rabbits' pain correctly. Alternatively, it may be that rabbits do show relevant facial signs of pain, and we have not yet identified them clearly.

Attempts to quantify the pain being experienced by an animal can be made by 'pain scoring'. This will often give separate scores to the overall appearance of the animal, the animal's food and water intake, the animal's behaviour when undisturbed and when provoked (e.g. site of pain touched) as well as clinical examination (e.g. the nature of the lesion). The use of such scores encourages regular close examination of the animals and can help to assess how well analgesia is working. As we saw just now, scales need to be developed and personnel taught how to use them. Moreover, researchers should use appropriate analgesia as part of their meeting the 3Rs, both during the procedure and afterwards. Module 32 outlines the pain pathway and the use of drugs at each point on it.

Slide 30:

Another aspect of minimising distress in animals used in research, i.e. refinement, is to assess their welfare.

This takes two forms. One is to assess the welfare impact of the proposed experiment. The other is to assess the animal's overall welfare outside the experiment, as you would with farm animals using the Welfare Quality® project framework.

Assessing the likely impact of a research protocol is challenging because the study itself may be a new procedure or approach, and the assessment has to be made in advance. However, researchers in New Zealand have developed an Animal Welfare Grading tool to assess the impact of research on animals' welfare.

The approach is based on the Five Freedoms, and estimates how long animals might experience any compromise in the freedom concerned, and how severe that compromise would be. There are five domains and each is graded from A to E (where A represents no welfare compromise in that area, B represents moderate compromise, and E represents very severe suffering). The next slide shows how this works.

Slide 31:

Mellor et al. (2009) give an example of using this grading system to assess the welfare impact of a study on the effect on under-fed animals of exposure to severe cold.

You can see from the slide that the first four domains of welfare, concerning the animals' physical functioning, were thought to be affected moderately by this relatively short experiment. However, 'mental state' has a lower grade than all the others because marked suffering is anticipated based on the extreme cold and under-nutrition, and the length of exposure to this.

Consequently, the overall grade for the anticipated welfare disruption caused by the study is D. It then becomes a matter of ethics and judgement whether the experiment would be allowed.

Regarding more routine assessments of animals in their housing, these have not been universally established for laboratory animals, but might follow the structure of the Welfare Quality® project framework developed for farm animals (mentioned above and described in Module 9). Particular issues of concern include the need to adjust housing and conditions for the welfare of animals with debilitating diseases or who are disabled in consequence of their experimental use. The earlier example of animals used in research on Huntingdon's disease illustrated that.

Slide 32:

We finish with a note about how to protect the welfare of animals used in research, testing and education.

Broadly, the regulatory approaches involve a competent authority who licenses the research premises and sets standards for the institution and the usage of animals within projects. Normally this means that there is also some legal protection, usually national; however, EU countries, for example, also have common international legal standards to follow.

Compliance with the standards and law set by the authority is then overseen by a national or local review committee or individuals.

Different countries apply this approach in three different ways, broadly.

One is centralised inspection. For example, in the UK, licences are dispensed by a government department at three levels:

- to the person conducting the research, allowing them to use animals for a specific purpose
- the second level is approval of the project itself
- the third licence is given to the institution if it meets necessary standards. Inspectors can visit institutions unannounced as well as by appointment.

There are criminal sanctions available to punish those who break the law, although these are rarely, if ever, used.

In addition, UK institutions must have local ethical review committees containing participants from outside the institution as well as scientific and ethics experts. Similar Animal Care Committees are used at universities in Canada and the USA but without the additional approval or licences directly from the government.

Brazil is another country that has centralised inspection. In 2008, the country enacted a law to protect laboratory animals used for research, and Ministry of Agriculture inspectors visit institutions. However, there was already a national animal care and use committee (CONCEA) in place, and those members have expressed concerns that the law is not strong enough and might limit their ability to do more. For example, the law does not refer to the 3Rs or specify permitted methods of euthanasia.

Slide 33:

Self-regulation is another form of protection. For example, in Australia, there is no centralised government administration or federal law, but there are codes of conduct. Meanwhile, each state has enforced self-regulation using local committees, under local state law. The committees have experts from within institutions and members from outside with interests in promoting animal welfare. The state laws usually incorporate some reference to a national code of conduct on care and use of animals in experiments. These codes incorporate the 3Rs.

Turkey has a similar system: a law was passed in 2004 and 2006 mandating ethical review committees – there is one central committee and 73 local committees.

The third type of protection is self-regulation, such as that found in the USA, where there is federal law to protect some laboratory species, and local committees within the research institutions implement the regulations. They ensure that guidelines on animal care are followed, and they have the power to suspend research.

Overall, core concerns about animal experimentation, such as preventing repetition, establishing the clear necessity of any research, or comprehensive promotion of the 3Rs, can only really be addressed centrally and with a culture of transparency. Variations in this approach are becoming the norm internationally, and the OIE guidelines provide minimum standards for countries that are just getting started in this area.

Slide 34:

This concludes today's lecture. Note that there is much we have not covered, such as the use of primates and details of how you might assess welfare in different species. However, you now have an overview of why we use laboratory animals, of the welfare concerns, including the role of the 3Rs and humane end-points, and how to improve animals' welfare in the laboratory and through the use of ethics review committees.

As a veterinarian, you may work in a laboratory or you may be an external member of an institution's ethics review committee. While you would need more specialised training to oversee animals in a laboratory, and more experience and knowledge of the ethical and welfare issues involved, you now have the basic tools and guidelines to help you if you work in a laboratory or serve on an ethics committee.