Mixing it up – ethics, science and adventure tourism

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Cam Reid Oration

Foreword

Early in 2014 zookeepers in Copenhagen killed a perfectly healthy young giraffe called *Marius*, and did so by captive bolt rather than by anesthetic so that he might be safely fed to the lions. This was done so as to avoid inbreeding among the giraffe population at the zoo. The whole process was made public (though sensitively) in the interests of transparency. About a month later the same zoo killed four lions.

The global protests reverberated. I am guessing each of us was momentarily disbelieving when we heard the news. Of course the authorities had very good answers for all of our obvious questions; they had been wrangling with these decisions for a long time.

A couple of years earlier, *Happy Feet* the Emperor penguin washed up on New Zealand's coastline a long way from home. He was nursed back to health, taken back to his beloved Antarctic waters by boat and released with a reliable tracking device. It transmitted for four days. It then fell silent. *Happy Feet* had, probably, been eaten.

No conclusion or contradiction is being offered or drawn here. But when that particular set of parallels was drawn by our after dinner speaker on the last night of the 2014 ANZCCART conference, there was much rubbing of chins.

As there had been for the whole conference, the use of animals for research and teaching can be awkward. Necessary, justifiable, ethical, well managed, well regulated without doubt. But still it can be awkward. It raises awkward questions. We all, especially Animal Ethics Committees, try to provide good answers to those questions.

What is 'awkward'? Killing *Marius* is awkward, I think. Releasing *Happy Feet* to the sharks is less awkward I think, but still somewhat so.

When I contemplate the awkwardness that, on average, I think the public feel towards the use of animals in research and teaching then the *Marius* story serves me well as a direct analogy. It helps me understand public unease.

ANZCCART exists both to defend the practice of using animals in research and teaching, and to strive for continuous improvement in that use. We like to learn how to do things better. I hope that the following pages achieve that. They are a record of a conference that, both by popular acclaim and by private critical feedback, was a success. The main reason for its success was, surely, the speakers. Here is what they had to say.

Pete Hodgson
NZ Independent Chair
ANZCCART

Scapegoats and tourists – science as the source of, and the answer to, ethical dilemmas

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Abstract

Humans cannot live without having an impact on other species. Animals and humans are part of an interdependent and interlinked system, both ecologically and socially. In terms of animal welfare, this system often comprises the animals themselves, as well as people in charge of animals, people with formal oversight of those in charge of animals, people with an interest in animals, and citizens. In this system those directly involved in the research are generally responsible for animal welfare. In addition, people at different levels of the system have understandings of animal welfare that may differ from those at other levels. Finally, there are some real dilemmas about which reasonable people can disagree, particularly in research, testing and teaching involving animals, where harms are used to produce benefits. If the diverse expectations, roles and responsibilities of the people who make up the different levels of the animal welfare system are important, then how good is society at acknowledging them and providing the confidence, resources and opportunities for those involved to undertake those roles and responsibilities?

Introduction

Dorothy's Funeral, Cameroon, by Monica Szczupider (http://ngm.nationalgeographic.com; http://www.monicaszczupider.com/).

Dorothy was in her late 40s, which is well into retirement age for a chimp, when she succumbed to heart failure. As caregivers at the Sanaga-Yong Chimpanzee Rescue Center bore her by wheelbarrow for burial, the typically boisterous apes rushed to the edge of their wired enclosure and fell silent. They stood – wrapping arms around one another, resting on each other's shoulder and not making a sound – as Dorothy's female keeper adjusted her head in preparation for a final farewell.... "It was unbelievably emotional. We were all struck." The chimps already knew the meaning of deep personal loss. All of those living at Sanaga-Yong had been orphaned when their mothers were killed by hunters. The chimps are prime targets in the illegal but widespread trade of providing African "bushmeat"....Dorothy was rescued from a hotel in Cameroon, where she was kept for 25 years on a chain. She was kept within view of Nama, another chimp, but the two could not touch one another. Tourists threw cigarettes, alcohol, and scraps of food to Dorothy. Despite her tortuous past, Dorothy was in fact one of the kindest chimps – or living creatures, in general – I have ever met.

This powerful image invoking feelings of sadness, sympathy, indignity, cruelty, respect, reverence, reminds us that we do not live without having an impact on animals. We not only interact directly by keeping and killing them, but also through disturbing their habitats, behaviour and ecological systems (Fraser & MacRae 2011). We also cannot live without animals; they provide us with food, companionship, protection, entertainment, learning opportunities, etc. This demonstrates the fact that humans and animals are socially and ecologically interdependent (Benton 1993). In considering the welfare of animals, then, it is necessary to think about the wider system, and our roles and responsibilities within it.

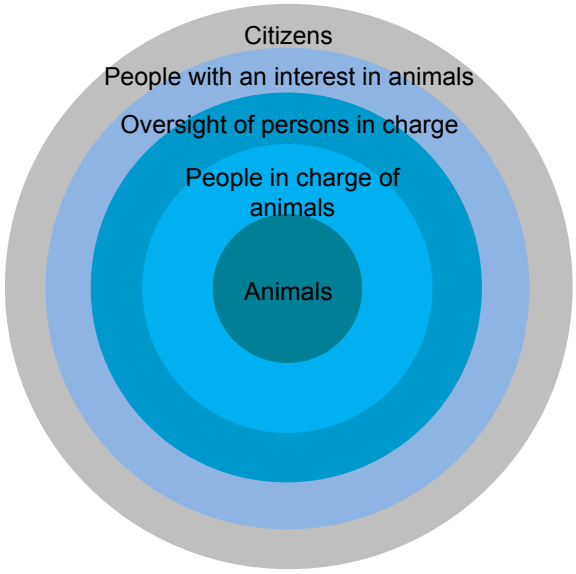
What is the animal welfare system?

At the centre of the system are animals. The system then includes persons in charge (e.g., farmers, pet owners, researchers, technicians and animal care staff); those with oversight of the persons in charge (e.g., animal ethics committees and animal welfare inspectors); those with an interest in animals (e.g., consumers of food and medicine, and animal advocates and activists). Finally citizens, who, while not necessarily having direct vested interests in animals, have a special role in the democratic process. There are many examples of the individuals and groups in society making up the animal welfare system and they can be thought of as actors arranged in concentric bands (see Table 1).

Arranged in this way, the system acknowledges that each group has a role, and thus a responsibility, for animal welfare. The schematic design also provides an opportunity to see, and question, some of the features of the system. First, costs and benefits tend to be borne differently. The benefits from animal use tend to extend outwards, while expectations for the care of animals tend to be directed more towards the centre. Does this mean that persons in charge of animals have a role as scapegoats having to justify what many others benefit from? Consider, for example, the view that the "livestock sector is largely to blame for our world being threatened by climate change, biodiversity loss, human health challenges and natural resources degradation" (Raphaely & Marinova 2012). If Kant

Table 1 The animal welfare system in New Zealand arranged as concentric bands of actors (a) and examples of some of the individuals and groups which make it up (b).

(a) Diagrammatic representation of the animal welfare system



(b) Individuals and groups comprising the animal welfare system

Animals
Research animals, farm livestock, companion animals, animals used in entertainment, education and conservation, pest animals, animals in the wild
Persons in charge of animals, including owners
Researchers, technicians, animal house staff, farmers and farm workers, strappers and jockeys, truck drivers, shearers, pet owners, animal shelter staff
Individuals and groups with oversight of persons in charge
Animal ethics committees, animal welfare inspectors, National Animal Ethics Advisory Committee, levy-funded industry good bodies, e.g., DairyNZ, Beef+Lamb New Zealand
People with an interest in animals
ANZCCART, media, New Zealand Veterinary Association, Federated Farmers of NZ, World Organisation for Animal Health (OIE), retailers, consumers, World Animal Protection, SAFE
Citizens
People with an interest in the public good

is correct in his assertion that "He who wills the end wills the means also" then have not all some sort of responsibility, including willing a change in the way animals may be treated (Midgley 1983)?

A second feature of the system is that, like tourists, individuals within each of the bands see the world from their own perspective in a varied but often limited way. Our responses to, for example, *Dorothy's Funeral*, reflect that perspective. Learning more of the features and expectations of others in different bands may act to change or reinforce our responses. Finally, given the number of actors and their diversity, and the dilemmas of using animals in research, testing and teaching we can see that science can be "the cause of, and solution to, all of life's problems" about which reasonable people can disagree. It is for this reason that, on behalf of society, animal ethics committees are required in research, testing and teaching, to consider these dilemmas.

In summary, we all have roles and responsibilities, share expectations, costs and benefits, and can disagree. This raises two important questions. How good is society at acknowledging those different roles and responsibilities? Does society provide the confidence, resources and opportunities to enable those roles and responsibilities to be best performed? These questions are now explored for three groups: young researchers; people with an interest in animals; and citizens.

Young researchers and the Three Rs mantra

The Principles of Humane Experimental Technique by Russell & Burch (1959) is not a 'bestseller' read. Nor are many of the abstracts in the 356 pages of the 8th World Congress on Alternatives and Animal Use in the Life Sciences held in Montreal in 2011 (http://www.wc8.ccac.ca/files/C17932_LivreCW8Abstract.pdf). ANZCCART's websites lack the detail required to really engage people, though *Animals in Science* (http://anzccart.rsnz.org/text/minimising.html) does list the following examples:

- **Replacement**: computer models; chemical models; charts; diagrams; manikins and physical models; mathematical and statistical models; use of plants; micro-organisms; cells derived from invertebrates; tissue cultures using cells from animals; videos of procedures conducted on animals to avoid repetition; voluntarily donated human tissues; and human volunteers.
- **Reduction**: assessment of the minimum number of animals needed; studying spontaneously occurring illnesses, injuries or phenomena of interest; using animals studied for other purposes, e.g., those already under anaesthetic or immediately after euthanasia; obtaining tissues from animals killed in abattoirs.
- **Refinement**: behavioural observations; sophisticated non-invasive imaging techniques; remote collection of urine and faeces; limiting sampling to that required to achieve the results; providing hormone implants instead of, for example, surgically modifying the animal to alter hormone secretion; keeping animals under general anaesthetic throughout the study then overdosing with anaesthetic to ensure they do not suffer or experience pain; use of anaesthetics, analgesics, sedatives and tranquillisers to relieve anxiety, fear, pain or distress; using experts, care and regular assessment to keep harms to a minimum; withdrawing animals from the study or euthanising them at that earliest time; gentle handling and extra attention to enhance well-being.

Collectively, this important information is hardly inspiring. Consequently, the Ministry for Primary Industries and ANZCCART are taking a fresh approach to the Three Rs by collating more engaging examples. For instance, how would you obtain a blood sample from a bird in the wild? What if the bird was nesting? How could we avoid risking nest abandonment? In contrast to shooting the bird, or capturing it in a mist net, one innovative method uses parasitic blood-sucking bugs placed in artificial eggs resembling the bird's own eggs in size and colour (Becker et al. 2006). An opening allows the bugs to feed on the birds. The bug's proboscis resembles a very fine needle and this, along with the secretion of a pain-reducing enzyme, results in little stress to the bird. The bug also secretes a substance that prevents blood-clotting allowing blood to be withdrawn from the bug with a syringe soon after its meal. This example of refinement (Figure 1) is designed to be short and engaging and will be available from ANZCCART.

Other examples are: *Safe and ethical seafood – an alternative to using mice in shellfish toxicity testing*; *Use*

Parasites as research assistants

Blood-sucking bugs as an alternative to syringes

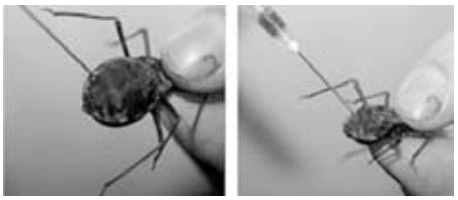
There are many reasons why we do research on birds. They range from understanding the impact of humans on nesting behaviour to protecting endangered species like Kakapo and Kiwi. Blood samples help us to understand wildlife. They allow us to monitor hormone profiles, energy consumption, physiology, genetics and more.

Taking a blood sample from a bird.

Taking blood samples from birds is very stressful, as we have to trap and handle them. This can lead to nest abandonment in brooding birds. Importantly, stress and its effects on the animal may change the information we are interested in.

How to collect blood from wild birds without too much interference?
One innovative method avoids handling altogether. This method uses parasitic blood-sucking bugs to take the sample. Mexican triatomine blood-sucking bugs are placed into artificial eggs resembling the bird's own eggs in size and colour. An opening allows the bugs to feed on the birds.

Triatomine bug in an artificial egg (photos by Christina Bauch).



Blood is extracted from the bug with a syringe.

The bug's proboscis resembles a very fine needle. This, and the secretion of a pain-reducing enzyme, allows the bug to feed with a minimum of stress to the bird.

The bug secretes a substance that prevents blood clotting. This allows researchers to extract blood from the bug with a syringe. Blood is removed from the bug soon after its meal (within 30 min). This prevents changes in concentrations of hormones, metabolites and other parameters we are interested in.

Fig 1 A section from a refinement example collated and presented in such a way as to engage and stimulate students and younger researchers to think more about the Three Rs.

your mouse – computer-assisted learning reduces animal use in teaching; and *Enjoying the summer while experiencing the winter – altering photoperiod to understand seasonality in deer*. Further examples introduce infrared thermography as a means of non-invasive assessment of animal welfare; the use of animal manikins to teach veterinary nursing students basic techniques such as intubation, drawing blood and cardiopulmonary resuscitation (CPR); and the search for replacement alternatives for the Draize Eye Irritancy test.

Do animal use statistics satisfy people with an interest in animals?

The use of animals in research, testing and teaching is one of our most enduringly contentious human-animal relationships. Contentious issues are arguably best explored by identifying the issues, providing information, and involving those with an interest in them (Korthals 2008; Fisher 2010). Within New Zealand, statistics of animals used in research, testing and teaching are published annually. This information, which frequently elicits public and political comment, reports that, for example:

- 54% of the animals used are drawn from farms;
- 89% of animals are normal or conventional (e.g., non-genetically modified);
- sheep and cattle make up the largest group (163,000) mostly with little impact on their welfare;
- rodents and rabbits (70,000) tend to be the animals most exposed to high impact or most harmful procedures;
- 49% of animals are used by commercial organisations;
- 58% are used for veterinary and basic biological research; and
- 70% are alive at the completion of the work and are retained, e.g., on farms.

Overall, in 2012, 301,964 animals were used, 7.6% less than in the previous year (National Animal Ethics Advisory Committee 2013).

While interesting and important, this statistical information does little to explain what animals experience or what happens to them. Nor does it provide any insights into the issues researchers and animal ethics committees grapple with. Failing to acknowledge these difficult but real issues leaves a vacuum where those with the most "shrill and dramatic" perspectives seize attention (Rollin 1996). Furthermore, it is contended that, for example, alternatives to animal use and steps taken to minimise animal numbers, pain, suffering and lasting harm should also be published with the empirical results to enable others to identify novel approaches, question their validity and improve them (Anderson et al. 2013). Failing to do so obscures the care and concern researchers and animal ethics committees bring to animal use. The future will undoubtedly see greater attention to openness and engagement, democratic decision-making and transparent reporting (Ormandy & Schuppli 2014) rather than reliance on statistics and examples of "kids saved by research".

Margaret Somerville's book, *The Ethical Imagination – Journeys of the Human Spirit*, begins by searching for a shared ethic. Somerville contends that we should begin by working through contentious issues – searching for the common ground in reason, imagination and even spirituality, and forgetting about polar or extreme views. Perhaps one aspect of that common ground is that we do not, perhaps cannot, live without having an impact on animals. It is clear from two examples from the ANZCCART Essay Competition run in conjunction with this meeting that there is an opportunity for dialogue to explore that common ground to ensure animal use is acceptable.

> *The truth is, I don't know any scientists who work with animals… our attitude towards the use of animals in science is not based upon fact, but upon feelings and fear*
>
> *… conversation will serve to bind science within society and determine the future of animal use in research and teaching*

Finding that common ground, and ensuring the dialogue required for shared understanding, is one of the more important challenges for future researchers, interest groups (including the media), and citizens. Perhaps it is a role for institutions such as New Zealand's National Animal Ethics Advisory Committee?

Going beyond "fit and feeling good": public understanding of 'animal welfare'

Animal welfare has been described as a wicked problem – difficult to describe, complex, changing, and subject to inconsistencies and political debate. This is partly because there are different understandings of animals' needs and the value of the benefits humans seek from animals. Consequently, there are varied understandings of animal welfare (Fraser 2003; Fisher 2009). Two of the most common are first, how the animal performs or how fit it is, and second, what it experiences or feels. Together these can be understood as whether the animal is "fit and feeling good" (Webster 2005). A third can be added reflecting the animal's nature. Good welfare is associated with a reasonably natural life carrying out natural behaviour in a natural environment. Many people, however, have other beliefs and discourse has become crowded with terminology (Table 2) reflecting expectations that extend beyond "fit and feeling good".

Table 2 Some of the more notable terms reflecting beliefs and expectations of animal treatment which go beyond the common and predominant understanding ("fit and feeling good") of animal welfare.

Term	Common understanding
Aesthetics	Beauty or the appreciation of good taste
Dignity	To be worthy of esteem or respect
Integrity	The state of being whole or undivided
Intrinsic or inherent value	The value an entity possesses in its own right, as an end in itself, regardless of its utility
Respect	To admire someone or something deeply, as a result of their abilities, qualities, or achievements; to have regard for the feelings, wishes or rights of others
Reverence	Deep respect for someone or something
Rights	A moral or legal entitlement to have or do something
Telos	An ultimate object or aim, the nature of something

If the way animals are to be treated is increasingly being understood in these different ways, then "fit and feeling good" may not necessarily fully reflect public understanding. Conversely, is understanding animal welfare as "fit and feeling good" based on a limited understanding of ethics? While more abstract and difficult to deal with, define, or legislate for compared to the more empirical understandings of animal welfare, they may nevertheless be important. If societal views reflect these more abstract concepts then, it is argued, animal ethics committees should include them in their assessments of experimental protocols (Röcklinsberg et al. 2014). Indeed, Swiss animal welfare legislation is based on protecting the dignity and welfare of animals (Gerritsen 2013).

The Swiss Animal Welfare Act (2008) defines dignity as:

> *... the inherent value of the animal, which is to be respected by anyone who handles it; the dignity of animals is not duly respected if they are subjected to stress which cannot be justified by overriding interests; stress involves in particular the infliction of pain, suffering or harm on animals, frightening or degrading them, profoundly altering their appearance or capacities, or unduly instrumentalising them.*

Under closer analysis, Switzerland has defined a violation of dignity as unjustified stress. Stress is then said to involve the traditional concepts of pain and distress captured in New Zealand's legislation, but also three additional elements: humiliation; interference with appearance or capacities; and excessive instrumentalisation (Schindler 2013). These additional elements are themselves arguably as difficult to understand and define as 'dignity' itself. They have led to more questions: Can an animal feel humiliated? Can we simply apply human standards to define humiliation? What is the standard to determine major interference with an animal's appearance or its abilities? What is acceptable instrumentalisation in contrast to 'excessive' instrumentalisation? What does it even mean to 'instrumentalise' an animal? The only thing clear is that there are no general answers to these questions.

Despite these difficulties, it would seem possible that the concepts of dignity and welfare need not be separate entities. Rather, as welfare is already an evolving, multifaceted concept, it is possible that it could be further expanded to include due regard for animal dignity, thereby encompassing the additional criteria.

It would be pertinent to at least acknowledge these different understandings and their influence on public expectations of how animals are treated. This is especially so if human-animal values are increasingly being shaped by interactions with pets, arguably members of the family, while interactions with 'real' animals are increasingly absent from modern life (see Fisher 2013). Reaction to something which is unpleasing to the eye or undignified, for instance the killing of healthy but unwanted animals, without reference to the remainder of the animal welfare system, risks expectations that are ungrounded in the "fit and feeling good" understanding of animal welfare. Furthermore, it could conceivably result in poorer treatment of animals and persecution of persons in charge of them, if such understandings are not fully cognisant of the nuances of the animal welfare system. It has been suggested that good citizens are those that pay their fair share, know of society's needs, are vigilant stewards for future generations and are compassionate (Sachs 2012), even outspoken, nonconforming and disinterested (Saul 1995). To not at least attempt to better understand these challenging perspectives is to risk not being good citizens.

Conclusions

Reflecting on the social and ecological interdependence of animals and humans there is a need to think of animals in research and teaching in terms of a whole system. Thought of in this way, people have different and important roles and responsibilities for the welfare of animals. Thinking in terms of the system, rather than as particular individuals or groups with personal or institutional interests and motives, requires cognisance of the whole system. This, combined with the systematic reflection of issues in the public sphere as a genuine and comprehensive concern for the interests and well-being of those who entrust the individuals and groups with their respective roles and responsibilities (Thompson 1999), may be the only real alternative to the combative nature of public engagement where participants can be viewed as 'tourists' or 'scapegoats'.

How well do we acknowledge and take our roles within this system? How well do we give each other the confidence, resources and opportunities to undertake our respective roles? How well do we

empower each other to produce an equitable system? Reflecting on these and other questions will require, it is suggested, a range of broad initiatives; for example, incorporating the principles of humane experimental technique into early career development. At another level, there is a need to consider providing more meaningful information on what animals experience and the issues the people involved with them grapple with.

Researchers and teachers continue to be some of the most scrutinised and contentious users of animals. There may be benefit in searching for the common ground and shared understanding, the expectations that people hold of animals and how they should be treated. Finally, perhaps the most significant opportunity to contribute to unravelling the dilemmas associated with benefitting from harming animals is challenging people as citizens, not just as members of interest groups, to understand and consider those common expectations.

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Do we really need codes of ethics?

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Abstract

Codes of ethics attempt to promote the application of suitably tailored basic ethical principles to a given workplace or practice. However, according to ethical particularism there is no special reason to think ethics can be codified. The moral landscape may be – and appears to be – messy rather than rigidly patterned. If this is true, then instead of following principles we should be encouraging the informed exercise of conscience on a case-by-case basis. In practice this means doing away with codes of ethics and replacing them with helpful lists of features that typically count for something ethically, but leaving it to the collective moral sense of the ethics committee to determine when and where they count. In this paper I explain and defend the merits of this approach.

I am very much in favour of ethics committees. Nothing I say here should be read as a criticism of ethics committees or of the quality of the judgements that they reach. Quite the opposite: I am arguing that ethics committees can do fine without a code of ethics. This is just as well because ethics cannot be codified; all codes of ethics are false and unhelpful.[1] That, anyway, is what I am going to argue here, though I stress that what I say is offered in the spirit of exploration and not certitude. My conclusion is going to be that ethics committees should get rid of their codes and replace them with a helpful list of features that typically count for something ethically, leaving it to the collective moral sense of the committee to determine when and where they count.

I will begin by outlining what I take to be four basic presuppositions of an ethics committee. I will explain why, happily enough, each one is true, or very likely true. I will then outline an assumption that ethics committees make, but do not have to: that ethics can usefully be codified. I will then explain why there is excellent reason to think that ethics cannot usefully be codified and that as a result every ethics code is false and misleading. Finally, and by way of conclusion, I will briefly consider what this implies where ethics committees are concerned.

One terminological issue before I start: I use the terms 'ethics' and 'morality' (and the related 'ethical' and 'moral', 'unethical' and 'immoral') interchangeably. Historically, the terms have always been used as synonyms and I will continue that tradition here.[2]

[1] In saying this I am expressing a view known as 'ethical particularism'. It has a growing number of defenders in contemporary ethics, though the philosopher who has done most to detail and defend it is Jonathan Dancy (2000, 2004). For a recent discussion of ethical particularism see Hooker & Little (2000). There are, note, different forms of ethical particularism and some degree of negotiation over exactly what it involves. Some particularists will not doubt disagree with some of what I am going to argue here.

[2] The term 'ethics' derives from the Greek 'ethos' (customs). Morality derives from the Latin 'mores' (customs). This is not to suggest that morality and ethics now mean 'customs', it is just to show that the two terms have always tended to be used to mean the same thing.

Four necessary presuppositions

1. Objective ethical norms

The first presupposition an ethics committee must make is that ethical norms are objective. That is to say, that the truth or falsity of a moral claim is independent of anyone's feelings or beliefs about it. Our moral feelings provide insight into what is objectively right or wrong, but do not compose the rightness or wrongness. For an analogy: the reports of our five senses give us insight into the nature of the physical world, but they do not compose the physical world.

There would be no point in an ethics committee if ethics were composed of our feelings and beliefs (if it was subjective, in other words). If morality is subjective then whether an act is right or wrong is wholly determined by the feelings, values or beliefs of the person who is performing it. All that would be required to establish the ethics of an activity is a tick box asking "do you approve of whatever it is you are about to do?"

Is the assumption of ethical objectivism a safe one? Yes, very. Subjectivism about ethics is grossly implausible (as should already be apparent) and has no serious defenders.[3]

2. Moral realism

The second, closely related presupposition is that ethics is real. To put it another way, ethics committees assume not just that the truth or falsity of an ethical claim is independent of anyone's beliefs or feelings about it, they also assume that some ethical claims are *true*. This is essential because if there are no ethical truths then discussing whether something is ethical or not would be akin to discussing whether Father Christmas beats his wife. Ethics committees must assume the reality of what it is they are discussing.

How safe is this assumption? Very. It appears to most of us (that is to say, billions of us) that there are moral norms. The burden of proof always falls on the person who says that things are not as they appear to be. Therefore, the burden of proof is squarely on the shoulders of the person who denies that moral norms are real. That burden has not yet been discharged.[4] So, until or unless we are provided with excellent reason to think otherwise, it is rational to assume that morality is real; there really is a moral dimension to the universe.[5]

3. Access

The third assumption is that informed, reflective people of moral sensibility have fairly reliable access to the moral landscape. That is to say, their moral sense or moral vision is tracking the moral landscape and is thus a fairly reliable source of information about what is or is not ethically permitted at any given time, any given place. Not totally reliable, but sufficiently reliable that the considered and corroborated ethical perceptions of a committee of such people have a decent probability of being accurate. An ethics committee must make this assumption for if our moral sense is assumed to be hopelessly unreliable then one might as well toss a coin to determine the ethics of an activity.

Is this assumption safe? Yes. It is true that ethically speaking not everything looks the same to everyone. The existence of ethical disagreement between disinterested parties testifies to this. However, it is easy to get a misleading impression of just how much ethical disagreement there really is. For instance, an awful lot of what one might take to be ethical disagreement is not really ethical at all. It is disagreement about the natural facts. Take the widespread disagreement over the ethics of capital punishment. Much (not all) of this is due to disagreement over whether capital

[3] There are more sophisticated versions of subjectivist theories of ethics, known as non-cognitivist views. These do have a small number of contemporary defenders. However, as their defenders will admit, ethics certainly appears objective and non-cognitivist views are only going to be plausible to the extent that they can in some way accommodate these objective pretentions.

[4] Ethical nihilism (a.k.a moral error theory) does have some contemporary defenders (see Richard Joyce's *The Evolution of Morality* for a recent engaging defence). All I will say here is that moral norms are just part of a larger normative landscape, one composed of the norms of reason, including the norms of epistemic reason (norms of epistemic reason are reasons to believe what is true). The problem with the case for moral nihilism, at least as I see it, is that it is really a case for nihilism about the entire normative landscape. Yet any such case is self-defeating, for any argument for anything is an appeal to normative reasons. So it seems to me that we must conclude, on pain of incoherence, that something has gone wrong with any argument for moral nihilism, even if we cannot pinpoint where.

[5] At this point many like to point out that there is widespread disagreement about what it is right or wrong, as if this is some kind of evidence that morality is not real after all. Yet disagreement can only take place against a background of agreement. For instance: if we disagree about how far away we are from Kansas, we agree that Kansas exists and is a distance away from us. Similarly, if we disagree about whether Xing in circumstances S is right or wrong, we agree that Xing in circumstances S has a morality.

punishment is a more or less effective deterrent than lengthy imprisonment. That is an empirical matter, not an ethical matter. Likewise, take the disagreement over the ethics of abortions. Much (not all) of this is due to disagreement over whether or when the developing foetus possesses consciousness. Again, that is an empirical matter and is not ethical.

So, there is not as much ethical disagreement as there first appears. Plus if the objective ethical landscape has a topography and can alter over time (just as the physical landscape can and does – and note, it is no less objective for this) there is less still. For it will then be the case that an act might be right if performed at one time or one place, yet wrong if performed in another place or time (holding other things equal). As a result divergent moral judgements across time and place are not necessarily indicative of the unreliability of our moral sense. Varying moral perceptions across time and space are, instead, a result of the variable nature of the ethical landscape.

If this is correct, then in fact the only kind of problematic ethical disagreement is disagreement that a) occurs at the same time, b) in the same place, and c) is not about the empirical facts of the case. There is not much of this. And its existence is not very problematic. It demonstrates that our moral sense is not entirely reliable. But we knew that already. Indeed, it is no assumption of an ethics committee that our moral sense is infallible. After all, if our moral sense was infallible there would be no need for a committee. One person of moral sensibility would do. It is to correct for the possibility of ethical misperceptions that we have a committee of individuals. So although the existence of ethical disagreement does indicate that our moral sense is fallible, nobody (and no ethics committee) ever assumed otherwise. Ethics committees assume fairly reliable access to moral truths, not infallible access.

4. Significance

The fourth assumption is that it is very important to comply with ethical norms. Ethics committees would be a waste of time and resources if complying with ethical norms did not matter. How safe is this assumption? Very. Indeed, most moral philosophers would consider it a conceptual truth that ethical norms are norms it is incredibly important to comply with. Ethical norms are not like the norms of a club or the norms of etiquette. They are not something you can take or leave. We all (all moral agents, that is) have weighty reason to comply with them irrespective of our interests. Happily then, the assumption that ethics matters is not seriously in dispute.

In summary: ethics is objective, real, detectable, and significant. These are all essential presuppositions of any ethics committee worthy of the name and happily they are all true beyond any reasonable doubt. However, there is another assumption that ethics committees make that is far from true beyond a reasonable doubt. That is the one to which I turn next.

Do ethics committees need codes of ethics?

Typically ethics committees have codes of ethics that they have to apply. A code of ethics is a set of principles that dictate that certain considerations or sets of consideration always count for something morally, and count in the same way. So, a code of ethics binds a committee: it forces the committee to be on the look-out for certain features and then forces the committee to take them into account in a certain way, regardless of whether they appear to the committee to have any ethical significance in this context.

Lying behind any code of ethics is an assumption that the moral landscape has neat, regular patterns to it, patterns that remain fixed across time and space (the principles describe these patterns). How safe is this assumption? Very unsafe, I think. Indeed, the evidence seems squarely against it, as I will seek to demonstrate in the next section.

Before I do that it would be as well to consider whether an ethics committee has to have a code in order to be able to function. I think the answer to that is a clear 'no'. The committee could just trust the moral sensibility of its members. That was the point in having a committee in the first place: to tap into the moral sensibility of a group of informed morally sensitive people. Why not just trust that sensibility then? Why bind the members to a code that tells them in advance what they must consider ethical and that was probably itself devised by a committee that had no greater moral insight than they do?

I think there are three main reasons why ethics committees feel obliged to have a code of ethics.

1. Anarchy.
   The first is a fear of ethical anarchy. Unless a committee has a code that binds it the committee might start making crazy, inconsistent ethical judgements.
   In reply to this concern, if you do not trust the moral sense of the committee then there is little point in having one. Plus by getting rid of a code the committee becomes regulated by its common moral sense. We have seen above that it is a safe assumption that our moral sense is fairly reliably tracking moral reality. So the committee is regulated; it is regulated by morality itself via the moral sensibility of the committee's members. That, surely, is preferable to a committee being regulated by a code of false principles?
   Regarding consistency: principles reflect an unduly narrow view of what is consistent with what. Principles assume that if Xing in circumstance S was once wrong, it must be wrong to X in circumstances relevantly similar to S today. Yet there is no special reason to think this is true (as I will argue shortly). So far from encouraging consistency, principles encourage people to see inconsistency where there is none.
2. Convenience.
   The second reason is that unless there is a code those who submit proposals will be working in the dark; they will not know how to tailor their proposals to make them ethical.
   However, you do not raise ethical standards by encouraging a tick-box attitude to ethics. That discourages ethical reflection. Doing without a code and instead providing, say, a list of features that typically count for something ethically means that researchers have to ask themselves the best and most appropriate question: "does what I am proposing to do seem ethical?" This is a far better question than "does what I am proposing to do tick this or that box?" It means researchers will have to exercise proper moral judgement and justify their proposals by appeal to considerations that appear morally relevant to them, rather than by appeal to an externally imposed code.
3. There are some true ethical principles.
   The third reason is the belief that ethics can be codified. Given that the point of an ethics committee is to discern the rights and wrongs of a matter, it makes sense to make it apply the principles of a code if the code approximates the true ethical principles. Even if we do not have the full code, applying those bits of it we do know will raise the probability of correct ethical decisions being made. This is the most deeply entrenched and philosophically interesting of the three reasons for having a code, so I am going to devote the next section to explaining why it is false.

Ethics cannot be codified

There is no special reason in advance of investigation to think ethics can be usefully codified. Upon investigation there appears to be plenty of evidence that it cannot be. To put it bluntly: we have tried to codify it. We have failed.

Here is the sort of reasoning that leads to the formulation of ethical principles. First, we note that some acts are quite obviously wrong to virtually all those of moral sensibility. Then we try and figure out what it is they all have in common aside from being wrong. If we manage to isolate a feature (call it X), we conclude that this feature, X, must operate as a wrong-maker everywhere it occurs. Bingo: now we have the basis for a principle. If an act has feature X, that feature pushes the act towards wrongness. That doesn't mean X automatically makes an act wrong if it is a feature of it. For there may be right-makers as well and perhaps wrong-makers and right-makers can turn up in one and the same act. This does not matter though, for the principle just says that X pushes any act that it is a feature of towards wrongness. It is a negative ethical presence wherever it occurs. The principle says "if an act has feature X, this counts against performing the act".

So, for example, let's say you notice that an awful lot of those deeds that are most clearly and distinctly wrong are deeds that seem to cause suffering. You reason "well, causing suffering seems to be a feature that all these obviously wrong deeds have in common, so 'causing suffering' must be a feature that operates as a wrong-maker". You also notice that an awful lot of morally required deeds seem to promote happiness. You reason that 'promotes happiness' is a right-making feature. Now you've got two principles. One says "the fact an act causes some suffering always counts against it: it is always a wrong-maker". And your other principle says "the fact an act promotes happiness always counts in favour of it: it

is always a right-making feature of an act". Obviously there are going to be acts that contain both features. For example: removing an abscessed tooth from an animal's mouth. That act is going to cause the animal some suffering. That counts against the act. However, it also promotes the animal's happiness because it frees the animal from future discomfort. That's a right-making feature of the act. So the act of removing the tooth has right-making and wrong-making features. The overall morality of the act is then a function of how these opposing ethical forces play out. That is typically left to our moral sensibility to sort out.

Anyway, that's typically how we arrive at and justify principles. That's 'principle-thinking'. It is very seductive. Indeed, it has dominated western thinking on ethics for millennia. The problem is that it is faulty. There is no special reason to think that just because a feature counted ethically in one case, it will in another (or that it will count in the same kind of way).

Take a non-moral example. I like the taste of stewed rhubarb. If custard is put on stewed rhubarb it tastes even nicer. In the context of rhubarb, then, custard makes a positive contribution. We might say it is a delicious-maker. And in fact, putting custard on lots of things (apple pie, banana, trifle) improves those things. But what if I reason "well, as in these cases custard clearly operates as a delicious-maker it must operate as a delicious-maker everywhere"? In other words, what if I formulate a principle: custard always makes things taste nicer? Well, that's pretty clearly false. Put custard on some fries and the custard makes a nice thing taste horrible. In the context of fries, custard operated as a horrible-maker, not a delicious-maker.

The lesson is, I hope, quite clear: what counts one way in one context may count in quite a different way in another context. Something that in one context made a positive contribution, can in another context make a negative contribution. There is no reason whatsoever to think matters will be different where moral features are concerned. Indeed, it is easy to think of examples that appear clearly to demonstrate that exactly the same applies. Sometimes happiness promotion is a right-making feature of an act, sometimes it is a wrong-making feature. For instance, it matters what the source of the happiness is. The happiness a sadist gets from torturing his victims is not a moral positive: it does not lessen to some degree the wrongness of what he is doing. It also matters who is being made happy. The act of helping Dr Mengele (Auschwitz's 'Angel of Death') escape prosecution and set-up a happy life for himself in South America promoted Dr Mengele's happiness. But, in this context this is a feature that operates as a wrong-making. It makes the act *worse* not slightly better. Mengele did not deserve to be happy.

Note, the lesson is not "come up with more complex principles" such as "happiness promotion is a right-making feature except when the happiness being promoted is the happiness of a wicked Nazi or a sadist". That is to have failed spectacularly to draw the lesson. The lesson is that we have no reason in advance to think that there is a fixed, patterned way any moral feature – or complex bundle of features – is going to behave. The lesson is: there is no reason to think that ethics is principled *at all*. There may well be rough patterns in the ethical landscape, but there is no reason to think that they *have* to be there or that they are indicative of more precise patterns, or that they are fixed across time and space. If you go to the beach there may be rough patterns discernible in how the pebbles are arranged. But there does not have to be: it would be foolish to think "there *has* to be a uniform pattern to how these pebbles are arranged – a pattern that is fixed and unchanging – it is just a matter of figuring out what it might be".

So, the first reason to be dubious about ethical principles (and the principle-thinking that leads to their formulation) is that there is no special reason to think there will be any. It would be ever so nice and convenient if morality was codifiable. It would be nice if pebbles on beaches were always arranged in fancy patterns. But, wanting something to be the case and it actually being the case are quite different. And assuming in advance that the pebbles on the beach will be arranged in fancy patterns is, as we can all recognise, just silly and unjustified. The same, I think, applies to morality.

There is also reason to be dubious about the usefulness of ethical principles. Ultimately any justification for an ethical principle is going to derive from the fact it delivers verdicts that accord with the deliverances of our moral sense. As such, there is something a bit perverse about following a principle. It is to allow the tail to wag the dog. Why not just cut out the middleman and trust your moral sense? Surely that's far more sensible?

The final reason to think that ethics is not codifiable is that no one has found the code. For thousands of years moral philosophers have been trying to discern a pattern to the moral landscape. The results are in. They have all failed, and failed badly. Immanuel Kant offered one single principle (the categorical imperative). However, it delivers numerous counter-intuitive verdicts (such as that lying is wrong even if that is the only way to save a friend's life) and does not allow for degrees of wrongness (killing someone for fun is clearly a lot more wrong than stealing someone's chair, for instance).[6] Bentham offered the utility principle, a principle that would deem it fully ethical to subject one person to an eternity of misery if that would be the best way of giving everyone else an eternity of bliss. To most of us it is clear that wouldn't be ethical at all. There have been many, many more attempts to come up with a code (more complex codes consisting in lots of principles, hierarchies of principles and so-on). Each one is ingeniously defended, but each one has failed. Each principle or collection of principles either insists that some clearly unethical acts are ethical, or else is so vague as to really amount to no more than an injunction to 'be ethical'.

Why is this? Why, despite thousands of years of effort, has there been so little progress in normative ethics? Why do we just have a big (and growing) pile of cleverly defended, but ultimately implausible theories? Is it because moral philosophers are stupid? Well, perhaps. However, an explanation I prefer is that the ethical landscape is not patterned. Nobody has found the code because there isn't a code to be found. That seems like the reasonable conclusion to draw after thousands of years of fruitless searching. It is the conclusion we would draw in any other area. So, it is time to conclude that the moral landscape is far more analogous to the physical landscape than tends to be thought. It has a complex topography that can and does change over time and according to location. Principles insist otherwise. Principles assume that the moral landscape is flat, uniform, unchanging across time. Yet on reflection there was never any reason to think the moral landscape is like that and the evidence is telling us that it is not. If you pay attention to the raw appearances then though you will see rough patterns of sorts, there is nothing very precise and fixed. Ethical principles are attempts to impose a rigid, unchanging pattern on something that is just not like that.

That is why, in my view, it is time to start paying attention to the appearances – to the deliverances of our conscience – rather than trying to force the appearances to comply with a pattern that we have become obsessed with finding. We do not need principles. We were all aware of morality long before we were ever told about any moral principles. Indeed, most of us do not have any. Do you have a code of ethics stuck on your fridge door? When you hear about some atrocity or witness someone being bullied, do you apply some principle and infer that what is going on is wrong or bad? No, of course not. You see that it is wrong. Nobody needs principles to recognise injustices. Principles come later. They are a diagnosis (and a faulty one). Just as you do not need to be able to diagnose flu in order to get flu, you do not need principles in order to be able to recognise what is or is not ethical, or to behave ethically.

Ethical principles seem to be a result of wishful thinking, of a desire that things be more ordered than they really are. This is not to say that we should slavishly trust our moral sense. As already acknowledged, our moral sense is not entirely reliable (none of our senses are!). That is why the views of others matter. They may see things more clearly than we do (one of the reasons why having a committee of informed, relevantly disinterested parties is important). Nor am I suggesting that our moral sense is free from the prejudices of our time and place. Adjusting for such prejudices is hard and I think there is no straightforward way of doing so. But principles are just as likely to be infected with prejudice. The justification for a principle ultimately comes from its ability to systemise the deliverances of our moral sense and thus, insofar as our moral sense is infected with the prejudices of our age the formulation of principles serves only to rigidify and engrain more deeply the prejudices in question. The best guard against prejudice, in my view, is to just pay attention to one's moral sense and that of relevantly disinterested others.

[6] Actually Kant's view allows for two degrees of wrongness depending on whether you can conceive of your policy being acted on by everyone at the same time (if you cannot, you have a perfect duty not to perform it) or whether you just would not wish it to be acted on by everyone at the same time (if you cannot you have an imperfect duty not to perform it). Violations of perfect duty are worse than violations of imperfect duties. Still, the point holds: there are more than two degrees of wrongness.

Applying this to ethics committees

If ethics is not codifiable what are the implications where ethics committees are concerned? Well, it means that having a code of ethics is a mistake. The code is going to be false and it leads to the wrong way of thinking about ethics. It encourages the thought that if a feature is a wrong-maker in one case one is committed (on pain of inconsistency) to judge it a wrong-maker everywhere. It encourages the thought that if we judged one thing wrong last time, we must judge a similar case wrong this time. Those are the thoughts that fuel principle-ism. If ethics is not codifiable then we have to learn to be more liberal about what is consistent with what. We have to unlearn the prejudices that principle-thinking has instilled in us. We have to learn to trust our moral sense and the moral sense of others on a case-by-case basis.

Rather than a code, my alternative would be a helpful list of features that typically count for something ethically and seem, most of the time, to count in a particular way. The principles of a code in effect already describe these features, but they say something far stronger about them. A principle says "this feature does count ethically and always counts ethically and always counts in the same way ethically". That, as I hope I have shown, is unlikely to be true. What is going to be true of most codes is that the features they highlight typically count for something ethically, and typically count in a particular way. The ultimate judgement about whether such features are ethically significant (and in what way) in the particular case under consideration is a matter of moral judgement and so rests with the committee. But that is a good thing: that is to allow the committee to do what it was set up to do.

Finally, what if I have been totally wrong above (and I am acutely aware of the possibility) and ethics can be codified? Well, we still do not know the code. So even if ethics can be codified we haven't figured out how yet. If we do not yet know what the correct ethical code is, surely the default should be no code, just a list of considerations that typically count for something ethically? That, it seems to me, is the sensible default in the absence of knowledge of the code. So, even if I have been wrong above, it is still a good idea to get rid of codes until or unless we find the right one.

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Openness in animal research: changing attitudes

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Abstract

Over past decades, the international scientific community has largely resisted speaking out publicly about animal research for fear of becoming the target of animal rights extremism or attracting unwanted negative attention. However, the recent launch of the Concordat on Openness on Animal Research, a voluntary agreement signed by dozens of major United Kingdom bioscience organisations, shows that improving transparency in animal research is becoming an increasing priority. This talk introduced the Concordat, discussed its background and objectives, explored the risks it raises and responds to, and posed the question of whether a similar initiative could be called for in Australia and New Zealand.

The Concordat on Openness on Animal Research (see http://www.understandinganimalresearch.org.uk/policy/concordat-openness-animal-research/) launched in the United Kingdom in May 2014, is a voluntary agreement signed by over 70 research organisations, including universities, medical research charities, pharmaceutical companies and professional societies.

It comprises four commitments, each underpinned by practical steps:

- Commitment 1: We will be clear about when, how and why we use animals in research.
- Commitment 2: We will enhance our communications with the media and the public about our research using animals.
- Commitment 3: We will be proactive in providing opportunities for the public to find out about research using animals.
- Commitment 4: We will report on progress annually and share our experiences.

The agreement was preceded by 18 months of public consultation and research into attitudes to animal research, led by a steering group established by the not-for-profit organisation *Understanding Animal Research*.

This documented widespread support for the use of animals in essential research, but uncovered areas where public awareness of legal and ethical constraints on animal testing remains limited. For instance, two-thirds of the British public did not know that it is illegal to use animals for research if viable alternatives exist, or that cosmetic research on animals is not permitted in the United Kingdom (following a 1998 ban).

As part of the practical steps outlined under the commitments above, the signatories have also volunteered to:

- make policy statements on animal research and provide clear information on involvement in animal research available on their websites;

- take steps to ensure their staff and students are aware of existing animal research;
- support and encourage researchers and staff to engage with media where possible, and identify spokespeople to talk about involvement in animal research;
- include information about animal research's contributions to scientific advancements or products in media releases about them;
- make examples of their progress in reducing, refining and replacing animals in research (Three Rs) publicly available; and
- include information about animal research in any talks or public events they take part in.

The idea of a public agreement on openness in animal research met with initial resistance behind the scenes at many organisations involved.

Typical reactions from stakeholders highlighted their concerns over risk to staff and students, a desire to avoid courting controversy, a fear of being singled out for negative reaction, concerns that it could put funding at risk and assertions that the public "don't want to know" about animal research.

When asked what were the key factors in the initiative's eventual success, staff at the United Kingdom Science Media Centre in London, who contributed to the Concordat's genesis and drafting as part of the steering group, emphasised the importance of patient negotiation over many months by many individual champions within different science organisations.

Recent positive case studies highlighting the tangible benefits of increased openness also played an important role. These included a decision by Oxford University to allow BBC journalists unfettered access to film inside its primate research laboratory, and sustained campaigns by the University of Leicester and Medical Research Council to increase transparency through outreach to journalists and the public.

The experiences of the researchers and institutions involved in the above showed that a commitment to openness demonstrates that animal research facilities have nothing to hide, effectively defusing the narrative behind would-be media exposés, and that proactive initiatives are far more powerful in this regard than reactive measures once negative publicity has gained momentum.

In New Zealand, public misconceptions about animal research were laid bare during a recent political debate over testing of novel psychoactive substances (or 'legal highs'). There is a real risk that limited public understanding of the essential role of animal research and the limitations of existing alternatives could lead to calls to limit or restrict funding to research involving animals, gaining momentum around another similar flashpoint issue.

An initiative to foster greater support for openness on animal research within institutions, similar to the Concordat, would lay the groundwork to mitigate this risk, but to be effective, it will require proactive steps before such a flashpoint occurs.

Statistics and ethics: how to appraise the statistical merit of a study without a statistician

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Abstract

Statistics is not a series of mathematical techniques, it is a way of thinking about the world. At the heart of statistics is experimental design – not complex calculations and a parade of Greek letters. For many research scientists the connection between statistics and their work is something of a mystery, a necessary nuisance on the road to funding and approval. The fundamental connection between statistics and ethics in experimental research is often poorly understood or completely overlooked.

The aim of any experimental researcher is to design a fair and competent trial in order to glimpse the truth about some fact of nature. This often involves the assignment of an experimental treatment to a group of living subjects followed by careful observation. Any such experiment which isn't underpinned by a carefully planned, rigorous experimental design is likely doomed to failure regardless of the elegant biology proposed or academic pedigree of the team members. Any experiment involving live subjects doomed to failure before it has even begun is an unethical one.

The ostensibly rational act of attempting to understand nature through experiment and observation is in fact plagued with bias and contaminated with random variation. Blinding, randomisation and an adequate sample size are the tried and tested elements of experimental design to minimise and mitigate these unwanted effects.

Experimental design is a complex subject but is underpinned by basically intuitive concepts.

Blinding and randomisation

The concept of blinding is a powerful design feature for minimising many forms of bias and outside interference in an experiment. It is important to think carefully about which team members need to be blinded: the person administering treatment; the carers/handlers; safety monitors; raters; the analyst, etc. It is not always possible or necessary to perform an experiment under blinded conditions; however, blinding should be the default setting. Another default should be randomly assigning subjects to treatment (randomisation). There are different mechanisms of randomisation, e.g., coin toss, random number tables, sealed envelopes, computerised systems, etc. Some are more prone to manipulation than others; careful thought should be given to the best method for a given experiment.

The key to good experimental design is to nip the potential adverse issues of bias in the bud by designing them out of the experiment. The additional effort required shows utmost good faith on the researcher's part and lowers the potential opportunity, temptation or even the appearance of manipulating the experiment. These features form an easily recognisable demarcation between quality (and ethical) experiments and the rest.

Sample size

An ethical experiment has adequate 'statistical power' – this is usually achieved through an adequate sample size. This is a perennial weakness and area of thorough misunderstanding in preclinical research.

The concept of statistical power should be basically intuitive. In order to detect, observe or measure something of interest (an effect) a scientist needs to use an instrument. Smaller effects require more powerful instruments to reliably observe them. This is not a difficult concept to grasp: larger more powerful telescopes are required to observe ever distant objects, larger more sophisticated particle colliders are built to detect increasingly diminutive sub-atomic particles, etc. By not matching the appropriate instrument with the anticipated effect of interest, the researcher runs the risk of not being able to see what they set out to look for.

A formal sample size calculation as part of an ethics application requires the researcher to think deeply and communicate clearly about their planned experiment and what the expected outcome is. It forces them to walk the tightrope between futility on one side (sample size too small) and needless waste on the other (sample size too large). Underpowered research has been described as comparable to looking for something in the basement without bothering to switch the light on.

Underpowered experiments are useless; underpowered experiments involving life are worse than useless, they are unethical. They expose live subjects to experimental treatments and exploit scarce research resources without ever having a credible chance of satisfactorily achieving their aims. The futility generated from widespread underpowered research represents a quiet ethical scandal.

The scientist's effort is primarily expended in recognising and rooting out error

> *The scientific method's central motivation is the ubiquity of error – the awareness that mistakes and self-delusion can creep in anywhere and that the scientist's effort is primarily expended in recognising and rooting out error*
> (Donoho et al. 2009)

The uptake and use of statistics and statistical thinking across different research domains is curiously variable. In the age of evidence-based medicine in clinical research it would be difficult to obtain funding, ethics approval, publication or peer acceptance without incorporating the fundamental concepts of blinding and randomisation and a properly justified sample size into the experimental design. This does not appear to be the case in preclinical research; these concepts are persistently conspicuous by their absence in ethics approval applications and scientific journal publications.

Experiments without blinding and randomisation are notoriously prone to false positive error, as they are easily manipulated, even subconsciously, to achieve a desired outcome. Conversely, underpowered experiments are prone to false negative error whereby evidence of efficacy goes unnoticed simply because the experiment was too small. When a poorly planned and executed experiment simultaneously unleashes these forces it is difficult to foretell what the observed result will be; however, it is unlikely to be close to the truth. Ethics committees have a unique role in ensuring researchers have worked hard to identify and minimise all possible sources of error.

The unique positioning of the ethics committee

The basic production line, or cycle, in scientific research can be thought of as follows: funding approval; experimental planning and design; ethics approval; study execution; and finally, publication. Adherence to good experimental design in clinical research is typically enforced by a combination of factors across the production line, including strong regulation, clear and rigid ethical standards, pre- and post-publication peer-review, and a general consensus across the clinical research community. Preclinical research seems to be sheltered from some or all of these forces. Similar to most scientific communities, change is unlikely to come from within. Trenchant criticism in the form of published review articles and editorials occur from time to time; however, these efforts are often too far downstream from the all-important planning and design phase to disrupt the production line and compel change. The ethics committee, on the other hand, has input early in the production line allowing far greater leverage to be brought to bear.

The path forward

Criticism and moralising are easy; coming up with feasible solutions to foster alternative behaviours is more difficult. An immediate and obvious problem is that not many ethics committees have access to a statistician or the requisite experience in the subtle aspects of experimental design to subject ethics applications to a thorough review. However, appraising the statistical merit of a planned study may not be as difficult as thought. Simple tools such as checklists, worked examples and re-formulating the ethics approval application form should be considered.

As a general heuristic it is often easier to identify instances of poor experimental design than good. The following examples could be used to rapidly identify poor design with little or no experience in experimental design.

Review: Blinding and randomisation

As a simple rule, if no mention is made of the concepts of blinding and randomisation then the investigators probably don't understand them or plan to ignore them.

Review: Sample size

For many reasons, preclinical experiments don't need to be as large as clinical ones. What is a cause for concern is often how poorly preclinical sample sizes are justified. Table 1 lists common but unacceptable sample size justifications which may be useful to guide committee members on what not to approve and why not.

As a general rule sample size justifications resting on practicality, previous experience, precedence, guesswork or cost and time constraints are commonly used to arrive at convenient, small and completely unethical sample size estimates.

Borrowing other designs

Another easy to spot error is "borrowing" the entire design from a published "successful" study. The logic seems to be "if it worked for them, and it was published, then it ought to work for us". This is in fact a very poor set of choices. Each experiment should be designed for the specific problem at hand. While it is a good idea to use the results (and discussion) from previous, similar experiments to inform the study design, previously published studies are liable to be un-blinded and underpowered. This seemingly rational design philosophy usually perpetuates poor design.

Table 1 Unacceptable sample size justifications.

"This experiment will require a total of 20 subjects in order to achieve significant results." This single sentence rationalisation is a surprisingly common example. There is no information whatsoever about what is being compared, how it is being compared nor what is the anticipated effect. What are "significant results"?
"Our sample size is based on a previous successful experiment by Lucky et al. (published in the *Journal of Inappropriate Prestige*) similar to ours where 20 subjects were used." "Borrowing a sample size" is not an acceptable justification. A prospective sample size calculation (ideally based on Lucky's findings) for the experiment is required with all relevant information.
"Professor PhD (3,000+ publications) has informed us 20 subjects are required to achieve significant results." This is indeed encouraging; however, the rationale and specific details of Prof PhD's estimates and workings need to be spelt out in the application. Regrettably these matters cannot be taken in good faith.
"Considerable previous experience in this field assures us 20 subjects will be sufficient to achieve significant results." See previous.
"Our previous (successful) ethics application had a sample size of 20 subjects; thus we plan to use 20 in this comparable experiment." Precedence is justification for lawyers not scientists. Each ethics approval requires a systematic and transparent justification of the sample size.
"With a cost of over $2500 per subject plus housing our grant will allow a sample size of 20 subjects." This is known as a pragmatic sample size calculation. It manages to be simultaneously honest and unethical.
"We choose to use only 5 animals to minimise loss of life." These are admirable sentiments. However, this is likely to be a completely futile experiment. An ethical sample size allows you to minimise animal use while simultaneously maximising the chance of success.

The poverty of *n* = 6

There is a curious, persistent folklore in preclinical research whereby six subjects per group is considered an adequate sample size for almost any planned experiment. This is underpinned by the logic "if you can't see an effect with $n = 6$ then it's not worth seeing". Table 2 may be useful in disabusing researchers of this convenient and cherished myth.

If you consider a simple design where at the end of the experiment two groups are compared and the endpoint is the proportion who responded to treatment, it can be seen that single figure sample sizes (per group) are capable of reliably detecting only a fraction of possible results (those demonstrating enormous treatment effects, e.g., say 90% response versus 10% response) and offer very little reliable insight in all other situations. Reliably detecting more subtle but still clinically relevant effects, e.g., 60% response versus 30% response, would be completely beyond the scope of the default $n = 6$ design. As a reminder the 'treatment' introduced by Dr Ignaz Semmelweis (hand disinfection with a solution of chlorinated lime) reduced mortality from puerperal fever from approximately 35% to 5% – this is considered an enormous 'treatment effect'.

Nudge

Another useful strategy may be to integrate nudge theory, originating from behavioural economics, into the ethics approval application form. Nudge theory argues that indirect suggestions can raise awareness and influence the motives, incentives and decision-making of groups and individuals at least as effectively as direct enforcement.

A nudge towards implementing blinding and randomisation could have the following form:

Blinding – *briefly discuss the implications of blinding in your experiment: …*
List all study personnel who are blinded: …
List all study personnel who are not blinded (discuss): …

Randomisation – *briefly discuss the implications of random treatment allocation in your experiment: …*
Describe the planned method of randomisation: …
If randomisation is not planned please discuss: ...

Table 2 Sixty-six outcomes from a 2-group experiment comparing the observed proportions responding to treatment. Cells indicate typical sample sizes required in each group for high powered research (80% power and $\alpha = 0.05$). Sample sizes of 6 per group or less are shaded grey.

% responding to treatment in group 2 \ % responding to treatment in group 1	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
0%	-	38	19	12	9	7	5	4	4	3	3
10%		-	195	60	30	19	13	9	7	5	3
20%			-	292	81	38	22	15	10	7	4
30%				-	356	93	42	24	15	9	4
40%					-	388	97	42	22	13	5
50%						-	388	93	38	19	7
60%							-	356	81	30	9
70%								-	292	60	12
80%									-	195	19
90%										-	38
100%											-

Improving the sample size justification might be nudged along as follows:

> *Does your experiment involve exposing live subjects to an experimental therapy? Yes/No*
> *If Yes – have you consulted a statistician? Yes/No*
> *If No – explain how you estimated the required sample size:…*

A self-scoring quality indicator could round out the section:

> *Start with 0:*
> *Add + 1 for blinding*
> *Add + 1 for randomisation*
> *Add + 1 for consulting a statistician*
> *Add + 2 for a coherent estimated sample size calculation.*
> *Report the total score:…*

The ARRIVE guideline

The ARRIVE guideline (Kilkenny et al. 2010) was developed for reporting the results of an experiment, but integrating its content into the ethics approval application form would also raise the bar and make an excellent guide to designing an experiment (e.g., Item 6 "Study Design").

Education

A well-known maxim in the field of quality management states: "Quality comes not from inspection, but from improvement of the production process". If the ethics committee is to raise methodological standards in experimental design, simultaneous efforts must be made to explain the motivation for this stance as well as encouraging education and up-skilling in the research community. This task will require clear communication, mutual understanding, education and delicate diplomacy.

The discipline of statistics and statisticians themselves must share a large proportion of the blame for the poor uptake and understanding of even the most basic concepts of experimental design in many scientific domains. A wise start may be to banish the word "statistics" where possible and replace it with "experimental design". Workshops in "experimental design" should be encouraged as well as more effort made to consult an "experimental design professional" at the planning and design phase, before ethics approval. The power of blinding can be easily demonstrated to students and new researchers in enjoyable and innovative ways such as taste tests. Similarly the futility of small sample sizes can be demystified and clearly demonstrated in real life simulations.

Science is a community activity; history has shown these communities can be surprisingly resistant to change (recall Dr Ignaz Semmelweis). Excellence is the accumulation of good habits and this will not happen overnight. The ethics committee needs to do what it can to create an environment where excellence trumps precedence without overstepping its core duties.

Recognising and rooting out error

Ethics committees should conduct an audit of their own decision-making processes and past approvals. What is the median sample size granted approval? How many un-blinded experiments were approved which could have benefitted from blinding? What proportion of previously approved experiments has translated into further developments and discoveries? Can the committee regularly get access to an experimental design professional or at least as an occasional consultant to help appraise complex and contentious designs on an ad-hoc basis? Do all committee members understand the concept that small, poorly designed experiments are likely doomed to failure before they even begin? Does the committee have a process for resolving violent disagreement within the committee? Recognising and rooting out error may not be a task solely for the scientist.

Hazards on the road ahead

It would be a lot easier and more peaceful to preserve the status quo. Raising standards will be a slow and difficult process; some hazards can be anticipated. Researchers may present applications similar to earlier successful ones and no longer get approval. This can engender tension within the committee and between the ethics committee and the rest of the wider research community. It is possible scientifically expert, but methodologically unsophisticated, funding bodies

will grant funds to an experiment which will not be considered large enough to gain approval from the ethics committee, leaving the researcher stranded. In academic settings the rate of approved experiments in entire departments may, temporarily, grind to a halt. Previously approved experiments that would stand no chance of approval in the new era may be ongoing, thus presenting a new ethical dilemma. These are challenges familiar in any situation when new policy is implemented. Clear communication, feedback and discussion with all stakeholders in combination with skilful diplomacy by the committee chairperson will be required.

Summary

In experimental research, ethics and statistics are deeply intertwined. This relationship is extraordinarily poorly understood (or overlooked) in many scientific domains. Scientific and ethical progress tends to move in fits and starts. Certain areas in basic and preclinical research are curiously lagging in terms of experiment design; these research communities may be comfortably unaware of this fact. Attempts to identify and communicate this issue and suggest improvements haven't had as much traction as hoped. This may be due to the fact that these efforts (usually in the form of published review articles, editorials and post-publication peer review) come long after the experiments are completed and thus do not substantially disrupt the preclinical research production line. The ethics committee occupies a unique station early in the production line and may be a more potent force for stopping poor quality research while simultaneously encouraging new behaviours. In implementing these changes the ethics committee should not wait around for the services of a statistician. The concepts involved are relatively straightforward, and simple tools such as checklists, guidelines and worked examples can get the process started, along with a few nudges in the right direction.

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Enforcing 'good practice', 'scientific knowledge' and 'law's (minimum) standards'

Fundamental principles of the human-animal relationship

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Abstract

There are a number of fundamental principles which are pivotal to understanding how the law governs people's responsibilities regarding animals. These principles are essential if people participating in animal welfare related areas are to maintain their credibility, and be effective and efficient, not only in demonstrating their own competence in current forums and debates, but also in persuading and influencing future developments involving the human-animal relationship.

An understanding of those principles demonstrates not only how the law deals with animals and the public's polarised opinions concerning animals, but also how concern for animals affects both animals and people.

The law is society's rule book of what is acceptable, and what is not. Essentially, the law applies responsibilities to people setting out standards of acceptable and unacceptable treatment. Considerable debate continues to occur regarding whether the legal classification of animals as property provides sufficient protection for them. Therefore, it is helpful to recognise that in jurisdictions that have established animal welfare law, legislation distinguishes between animate and inanimate property by recognising that animals, as animate property, experience "pain and distress".

The subject of animal law has three essential criteria. Beyond the obvious first element (i.e. law that deals with an animal), the second element of animal (welfare) law recognises that animals are animate, and thirdly recognises the link between human and animal interests.

The legal definition of animal welfare

Despite many attempts at defining animal welfare, there is no one universally consistent and accepted definition. Irrespective of personal or professional opinions, it is critical for all those involved with animals to be conversant with the legal definition of animal welfare because it is the law, and the law's legal definition of animal welfare, that applies responsibilities to people regarding animals, and liabilities for failing to comply with those legislatively established standards.

Reference to the purpose of animal welfare legislation, and the wording of the offences, sets out that there are four key words that form the responsibility that applies to persons in charge of animals. Those four words are pain, distress, unreasonable and unnecessary. Putting those words together establishes the responsibility of people to prevent animals from experiencing pain or distress that is either unreasonable and/or unnecessary. These synonyms are captured in common terminology associated with animal protection/welfare law including, for example, the terms "humane", "protection", "anti-cruelty" and "welfare".

Good practice, scientific knowledge and minimum standards

Having established this responsibility, the law provides references to good practice and scientific knowledge to determine whether the animal experiences pain or distress, and whether or not the pain or distress is unreasonable and/or unnecessary[1]. In theory, this provides assurance that the standards are set in a way that references empirical, robust and reliable science. In practice, however, it is clear that not all scientists agree, and the evolution of scientific assessment demonstrates that the credibility of scientific methodologies and reporting can vary significantly.[2] In reality, while the law frequently refers to, and is guided by, science it is also heavily influenced by practicalities associated with the economy, the environment and society – including politics and politicians.

It makes sense that society's rule book regarding what is acceptable, or not, reflects the predominant opinion of society itself. Consequently, there are two key influences on the evolution of animal welfare law. The first is science – particularly science's ability to understand the animal's experience. But understanding the animal's experience is only half of the equation. The other key influencer shaping society's attitudes is technology, which enables the learnings gleaned from science to be communicated to the mass public. Communication and science have, in tandem, shifted the average person's understanding of what it is the animal experiences. In turn, it might be said that society's view – and consequently the law's view – of cruelty turns on what science and technology have educated the masses.

Utilitarian governance

Dr David Bayvel is recognised for frequently stating: "not all stakeholders are steak eaters"– but the reality is that most of them are. As the socio-economic status of a population increases, so too does their demand for meat protein. The United Nations Food and Agriculture Organization (FAO) predicts a 60% increase in demand for meat, milk and eggs by 2050. But it's not just a demand for animal products – there is a demand for food products that are safe and obtained in a way that is both humane (in respect of the animal utilised) and sustainable.

"The public" is obviously frequently identified as the consumer who is the target audience of other stakeholders, which include the Government, non-government organisations, the environment, the producers – and, of course, the animal. Each of these stakeholders has its own interests and currency, and many of those varied stakeholder interests are either competing or conflicting both in terms of objectives and methodologies. Non-government organisations frequently advocate for what they view as an improved life experience for the animals, exemplifying an innate value philosophical approach to questions of animal worth. In contrast, industry is largely attributed with applying an understandably commodity-based value to commercial operations which raise animals for food and/or other animal products. Government has a responsibility to balance and appropriately prioritise each of these competing and potentially conflicting stakeholder interests, with a view to determining and deciding what best serves the public's interest.

In the exercise of governments balancing and prioritising the interests of multiple stakeholders, the animal is one, but not the only, stakeholder in a utilitarian people-centric society where there are polarised opinions regarding the role of animals, and 'shoulds and should-nots' about human responsibilities regarding animals. The utilitarian system of governance (which seeks to be as inclusive as possible regarding the stakeholders and their interests) therefore functions on a legal system which distinguishes minimum legal standards from standards of best practice.

Conclusion

The basic tenet of animal welfare law – that is, that people should not treat their animals in a way that causes them unnecessary or unreasonable pain and/or distress – has remain unchanged since the inception of animal protection law over 200 years ago. In essence, the only thing that has changed is society's understanding of the animal's experience, and therefore society's view of what constitutes acceptable, or unacceptable, animal treatment. In a society where only a minority of people are truly conversant with the realities associated with the uses

[1] Section 10 of the New Zealand Animal Welfare Act 1999.

[2] See papers presented at the 2014 ANCCART conference.

of animals, in particular using them for research or for food, there is a greater divide between stakeholders' perceptions and realities. This is the environment in which governors have an even greater responsibility to ensure that the evolving standards of "good practice" and "scientific knowledge" are reflected in standards which not only reflect the dominant societal attitude, but which are also practical, realistic and enforceable.

These fundamental principles and concepts are expanded on in a book written by Ian Robertson and due to be released by the publisher Earthscan in 2014. The book "Animals, welfare and the law" facilitates people's ability to objectively, practically and authoritatively critically assess uses of animals in human society. The book sets out the fundamental principles which are essential to understanding how the law – as society's rule book – dictates and governs human responsibilities regarding animals, and people's business and/or personal interests which involve animals.

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A taxonomy of zoo ethics: welfare and continued life

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An ethical zoo

The modern zoo should be a powerhouse of conservation and a responsible zoo ensures that every specimen within its purview has a role in the goal of conservation, directly through meta-population management, and indirectly through education, advocacy or research.

A good zoo need not be shy in extolling its virtues: Zoos Victoria represents itself as a 'zoo based conservation organisation' and Taronga Zoo promotes its role in 'connecting people and wildlife', both unequivocally laudable pursuits. What gets lost in this promotion is that zoos primarily must be ethical institutions.

This is because a zoo is run by humans and humans, being autonomous, act as moral agents and most people would agree that the zoo has a moral responsibility to behave ethically when making decisions affecting its moral subjects, the animals in its care. The laudable pursuits are not themselves sufficient to make zoos ethical because there is more to an animal than its utility in human pursuits, whether conservation, new science, or the improvement of children.

Perspective

When talking of a zoo's responsibilities to animals in its care it is useful to recognise an animal perspective and a human perspective. To characterise these perspectives we can talk of 'interests' and 'values', the former relevant to animals as moral subjects and the latter relevant to humans as moral agents. In this context interests are the subject of science whilst values are the subject of philosophy and recognition of this dichotomy should help humans identify their responsibilities to animals in their care.

The modern debate on the ethical use of animals, particularly our responsibilities to their care, has been much influenced by Singer (1975). In Singer's utilitarianism the utility is suffering, and speciesism, like racism and sexism, "does not supply a valid reason to deny a sentient being a decent life free of suffering". Rachels (1990) argued to a similar end but based his concern on notions of equality: if suffering is wrong for people then it is a matter of justice that it is wrong for animals with the same capacity to suffer.[1]

Cochrane (2007) uses an 'interests' based approach to discuss the rights an animal might have in firstly, not suffering, and secondly continuing to live. It is useful to examine this approach because it attempts to address both the issues relevant to our discussion, those of welfare and continued life. It also relies heavily on 'time-relative interests' (McMahan 2002, 2008)[2]. It is also relevant because Cochrane takes it that animals can possess rights and accepts the

[1] For many philosophers, suffering was not a moral issue because animals felt pain but because it was instrumentally bad for humankind. And indeed, there are numerous studies which reveal that cruelty inflicted on animals can lead perpetrators to inflict similar cruelties on their fellow humans. A recent advertising campaign by the RSPCA drew on this, featuring the bruised face of a child with a stamp reading, "Tested on Animals".

[2] McMahan elegantly discusses interests in continued life in McMahan (2008) but for the relevance of psychological continuity within a life the primary reference is McMahon (2002: 39-43 and 69-82).

deontological commitments of a 'rights' based theory. This is broadly in line with the position adopted by zoos and zoo associations, that is, that animals in care have a right to good welfare and that this right imposes a duty on the holding institution.

The idea that we might talk of interests amongst sentient animals makes intuitive sense: for example, the idea that a sentient animal has an interest in whether or not it suffers or is happy seems like a reasonable thing to say. Note that an interest is prudential, that is, it relates to how things are for an individual. It relates specifically to an individual's well-being and for our purposes we can establish that an individual has an interest in something if a change in that something changes the answer to "how's it going?"

Interests can thus be distinguished from values which are judgements: for example, it might be said that something is beautiful (of aesthetic value), rare (of conservation value) or even the right thing to do (of ethical value). In making such judgments, however, we do not need to make reference to how things are for the individual itself.

A second point that needs to be made about interests is that they are intrinsic or instrumental. An intrinsic interest is an interest 'for its own sake', that is, an intrinsic interest has a direct effect on well-being. This intrinsic interest is counterpointed by an instrumental interest. An instrumental interest is secondary to an intrinsic interest. The instrumental interest is important because it impacts on an intrinsic interest but it is an interest 'for another interest's sake'. In Figure 1 the intrinsic interest is 'welfare' and there are two instrumental interests, those of 'health' and 'choice'. Good health and preferred choice are instrumental because they can have an impact on the intrinsic interest of 'welfare', but are not of themselves the answer to the question "how's it going?"

Interest in welfare

Zoos focus on 'evidence-based' welfare. This is the scientific evaluation of 'how things are going for the animal' and how things might be improved. This prudential view, wherein welfare is just about the animal's interests from the animal's perspective, continues to drive zoo discussions on responsibilities to animals in zoo care.

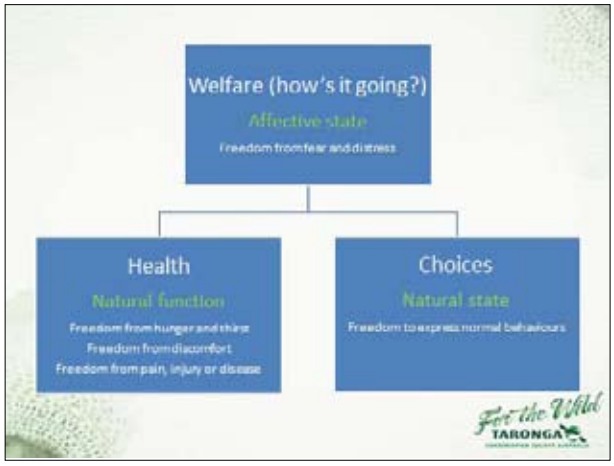

Fig. 1 Welfare and its instruments (Stamp Dawkins 2008), three welfare paradigms (Fraser 2009), and the five domains familiar from production animal ethics (Farm Animal Welfare Council, UK; Mellor & Stafford 2001).

Welfare relates to how an animal is feeling and for our purposes we follow Stamp Dawkins (2008) and consider feelings a consequence of the two stimuli, health and choice. Dawkins (2008) describes the two instruments of welfare by questions as in "how goes health?" and "how goes choice?". Implicit is recognition that affective state is the response to the stimuli 'health' and 'choice', the former in the sense of 'are you well?', the latter in the sense of 'doing what you want'.

Within these we fit the three welfare paradigms commonly referred to in the literature, those of 'biological function', 'natural state' and 'affective state' (Fraser et. al. 1997; Fraser 2009) which roughly translate as 'physical health', 'behavioural choices' and 'feelings'. Though not mutually exclusive these three welfare paradigms represent three quite different perspectives on the concept of welfare and most approaches to welfare have viewed the issue through one or a combination of these prisms.

There is a good fit with freedoms and domains, in particular the 'Five Freedoms', still a widely referenced benchmark for animal welfare. Though the Freedoms have come under criticism for addressing suffering rather than happiness and in some spheres have become constructively reinterpreted as the 'Five Domains' (Mellor & Stafford 2001), they still sit within paradigms: the first three, freedom from hunger and thirst, freedom from discomfort, and freedom from pain, injury and disease, all relate to biological

function; the fourth, the freedom to express normal behaviour, is natural state; and the last, the freedom from fear and distress, is welfare itself, affective state.

Note that we have already established that welfare is prudential, that it relates to how things are going for the animal itself. Thus the choices that must be offered to a human socialised animal are not the same as the choices demanded by a wild member of the same species: choice must address both *telos*, the 'gibbon-ness of gibbon', and individual particularities, whether the latter are a product of environmental history or the individual variation found within species. This has obvious implications for the practice of hand-rearing socially complex species such as primates and parrots. Individuals of both species might be perfectly happy in human company but experience distress amongst their own kind: thus once hand-raised the commitment to human engagement must be permanent. But note that once health and individually appropriate choices have been secured, responsibility for affective state ends; no carer is responsible for making the grumpy less so.

Interest in continued life

Having established that animals have an intrinsic interest in welfare Cochrane takes an interest-based approach as to whether an animal has an interest in a continued life. In doing this he draws heavily on McMahan. McMahan's thesis is that an animal with a potential for happiness has an interest in being alive to enjoy that happiness. He then argues that the strength of that interest is a function of both the capacity for a good life and the degree of connectedness between the animal now and its expectations of a good life in the future (McMahan 2002: 70).

There are a couple of considerations to bear in mind when we reflect on this concept: first that an interest is prudential and relates wholly to welfare which is about how an individual is feeling right now, and second that interests can be intrinsic or instrumental.

Hark back to Cochrane's definition of interests as a measure of 'how things are going': the instantaneous nature of the answer to the question "how are things going?" dismantles the distinction between an interest in welfare and an interest in continued life that the concept of 'continuity' tries to introduce. Cochrane established that an individual might reasonably have an intrinsic interest in welfare but this does not necessarily translate into any kind of interest in continued life.

The average human lives a life untroubled by concern of imminent and random death; should death happen in a random and unpredictable manner, and the human is dead in an instant, the answer to the question "how's it going" changes from "well" to no answer at all. The welfare of the person changes from something to nothing in an unconsidered moment and instantaneous death is therefore not a welfare issue to any animal. Should the death be foreseen, however, and mortal threat lived with, an instrumental interest in continued life would lead to suffering for the individual fearing death.

The human interest in continued life is not on account of future experience but entirely on account of current welfare. Even in humankind we might treat interest in continued life as an interest in instantaneous welfare. To plan, to have goals and projects to pursue, is good for my affective state right now as I contemplate them. The thing that gives humans an interest in continued life is the immediate effect on welfare of contemplating not being able to indulge the future projects. There is a critical difference between welfare (being suffering and happiness) and death. In the former the animal feels something, as do humans, but in the latter, obviously, it does not feel like anything for any animal, human or otherwise, to be dead.

Now consider that a gibbon, known to be able to mentally map its territory and visit fruiting fig trees on a schedule, might be able to anticipate tomorrow's fig and even take pleasure in that contemplation. But whilst this taken pleasure might be positive whilst it is taken, it does not mean that this indicates anything more significant than a positive welfare experience resulting from contemplation, exactly as it does for a human. A gibbon might get pleasure from eating a fig, and with a mental map of fig trees in its territory might get pleasure from the contemplation of eating a fig, but this does not give it an interest in the future. It actually improves the 'how's it going' now, that is, 'continuity through time' reflects a welfare interest not a continued life interest.

In short, it might be reasonable to talk of interests in the context of now, and for humans, and perhaps some animals, that interest in now might include, for better or worse, a degree of anticipation, or memory. But unless the animal has the human capacity to

contemplate not being alive for anticipated goods (however described), death is both irrelevant to current welfare and thus clearly irrelevant to an interest in continued life.

There is nothing in the natural world that suggests any non-human animal has a capacity to have 'thoughts about thoughts'. Sentient animals "may all have the capacity for conscious experience and may also possess desires, but there is little in their physiology or behaviour to suggest that they have the ability to reflect on their own thoughts" (Cochrane 2009: 667); they cannot be considered autonomous in the 'Kantian' sense.

It is therefore the manner of death and not death itself that an animal has a stake in. When an animal is in the company of animals that communicate discomfort or fear there is no doubt that their answer to the question "how's it going?" would be "not that well". In this sense it seems reasonable that a sentient animal might have a response to what is happening to other animals around it: in this context a sentient animal might react as though concerned by death but on account of an interest in welfare, not an interest in continued life.

The ethical argument for eating kangaroos that have been killed with a bullet to the head in a spotlight at night turns on the fact that death is instantaneous. That is, there is no opportunity for an animal to suffer because the behavioural stimuli it receives from its neighbours are not negative and the individual itself does not anticipate the bullet. The following morning it is possible one of the mob will ask "where's Skippy?" but Skippy has not had its interests compromised.

It is no different for humans. I might have a tumour that will kill me in exactly six weeks. Let us suppose the tumour does not impact my health and I am unaware of it. The tumour will impact on my continued life but as far as I am concerned it will have no impact until I am aware of it, at which point I will worry and my welfare will be compromised. Thus I have an instrumental interest in continued life that rests on my capacity to reflect on imminent doom.

That evolution is a parsimonious process is ignored by philosophers yet if an animal has an interest in welfare its fitness is not improved by an interest in continued life. The accident that is humankind was an unlikely series of events and the kind of contemplative capability that is required for an animal to have an instrumental interest in continued life is clearly and predictably beyond the capacity of any non-human animal. We can be surprisingly confident of this: any animal with the 'Kantian' autonomy referred to by Cochrane that underwrites reflection and reflection upon reflection, would surely communicate its ability. To assume that there are non-human life forms on our planet capable of autonomy but not choosing to let us know is perverse in the extreme. It is only in fiction that the white mice run the show but choose to say nothing.

Implications

That a sentient animal has an intrinsic interest in welfare but only a human has an (instrumental) interest in continued life does not mean that life is without value, rather that value cannot be based on an interest. This line of thinking has several repercussions.

First, McMahon's argument that "animals must have an interest in living to experience the goods that lie in prospect for them" (McMahon 2008: 67) because otherwise there would be no excuse for causing any suffering, even short term, cannot be a valid claim. There are no difficulties here because we can recognise value in animal life (and therefore justify short-term suffering) without recognising individual interest in continued life.

This uncoupling of welfare and death talks to the intrinsic value of life. That it is wrong to capriciously kill is because life has intrinsic value not because welfare is impacted by a humane death or because an animal has an interest in continued life. When euthanasia is described as 'cruel' there is a misunderstanding of the oxymoronic kind: cruel implies suffering and by definition euthanasia is not suffering.[3]

That an animal might have some kind of value is obvious: it might, for example, have value as a draft animal or as food. That it might have an intrinsic value, that is a value in its own right, independent of its utility, is perhaps less obvious. Indeed, we'd have to acknowledge that some animals might be considered of intrinsic value in one culture but completely disposable in another; cows, for example, have intrinsic value in India amongst Hindu but

[3] Note that the etymology of euthanasia is good-death but that it has more recently become associated specifically with death to end suffering. It is used here to mean good in the sense of sudden and without room for welfare concerns to materialise.

their lives, rightly or wrongly, are disposable in most other countries.

For the most part though, there is a growing acceptance that animal life has some value in its own right. And if we accept this intuition we must establish a way to value it, preferably a way that meets other intuitions, that, for example, we value it fairly and as objectively as we can. The proposition here is that we value it in much the same way that we value our own lives, that is, with reference to life history.

Second, the fact that interest-based rights can apply only to the sentient leaves most living things outside of any kind of consideration. This cannot be correct. The insentient struggle for life as tenaciously as the sentient. To value according to life history recognises that the insentient have valuable lives too.

Third, we must develop an approach to aggregated good. In democratic human societies the individual's life is indulged ahead of the collective. The individual animal could be valued as it is valued in our species but what is 'good' in humankind might not be 'good' in other species. It is fairer and respectful to value a life as it values itself; some species thrive under stochastic culling, in others the individual is of significant intrinsic value.

Respect for an individual should be something about 'it', not simply about us. There are several advantages to this approach: first, it introduces a degree of objectivity that insulates welfare from changes in cultural value; second, it seems intuitively fair; and third, it unhooks respect and welfare, thereby opening the possibility of respect for the insentient.

Fourth, much of the difficulty associated with weighing the moral aspects of welfare and death is because the two are not fungible: one is an interest and the other is a value. There is only one way to regard interests and that is through the perspective of an individual animal. There are myriad ways to attribute value, including one that is intrinsic, as we have seen. Prioritising interests and values is difficult though in the context of an animal in human care and taking a 'rights' based approach we can at least start from the position that an animal has a right to good care because it has an interest in good care. This should at least start to frame the 'animal rights' and 'environmentalism' debate because it establishes that arguments on 'right to life' are about people and their prejudices and not about animals.

Fifth, the recognition that life has an intrinsic value yet that value still has to be attributed obviously requires some philosophical context: at first sight it looks contradictory to have to attribute what is intrinsic. The use of 'attributed' and 'intrinsic' with regard to the value of an individual life are in this context synonyms. The intrinsic value must be attributed because there is no external agency prescribing it, but if it is an innate sense, comparable to or a consequence of, empathy, then the two terms become interchangeable, indeed indistinguishable, in the human mind.

Depending on the audience the relevance of life history probably needs more or less justification. For those who recognise the fundaments of morality in fairness and empathy, both traits common to social primates and clearly of evolutionary origin (Waal 2009), the proposition that life history might define good is probably obvious in that it seems fair, objective and respectful. This could be an intuition that draws upon Natural Law theory derived through evolutionary biology rather than an external agency. In this conception of Natural Law the intrinsic value of an animal's life should be informed by life history because it is to evolutionary biology that we must look for our ethical framework.

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Animal care in a 21st century zoo

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Excellent animal care is the raison d'être for zoos. To my mind this is one of the defining differences between good zoos and bad zoos worldwide. In the 21st century compromised animal welfare in zoos is unacceptable.

As the current President of the Zoo and Aquarium Association (ZAA) Australasia I am extremely proud of our members in regard to the life worth living they provide for the animals in their care. This is an area of developing and ever changing science and a 21st century zoo must be leading the ethical paradigm for animal care. As a zoo community we are sometimes criticised for having 'captive' animals – this criticism presumes 'captivity' is a bad thing and that the wild is somehow the 'holy grail'. This is not always the case.

But in saying that I also believe that zoos and aquariums need to be sure they are doing all they can to ensure a life worth living for the animals in their care – whether that be for the animals who live at the zoo or the wildlife that is cared for in zoo hospitals before release back to the wild. Animal welfare or best animal care is expected by our communities and without it zoos and aquariums are open to major criticism by society.

As a zoo and aquarium community in this region we have committed to the UN Five Freedoms in an animal welfare context for many years now. However, we have not really monitored this to the degree we should among members and consistency is not always guaranteed. There are also some important difficulties with this system.

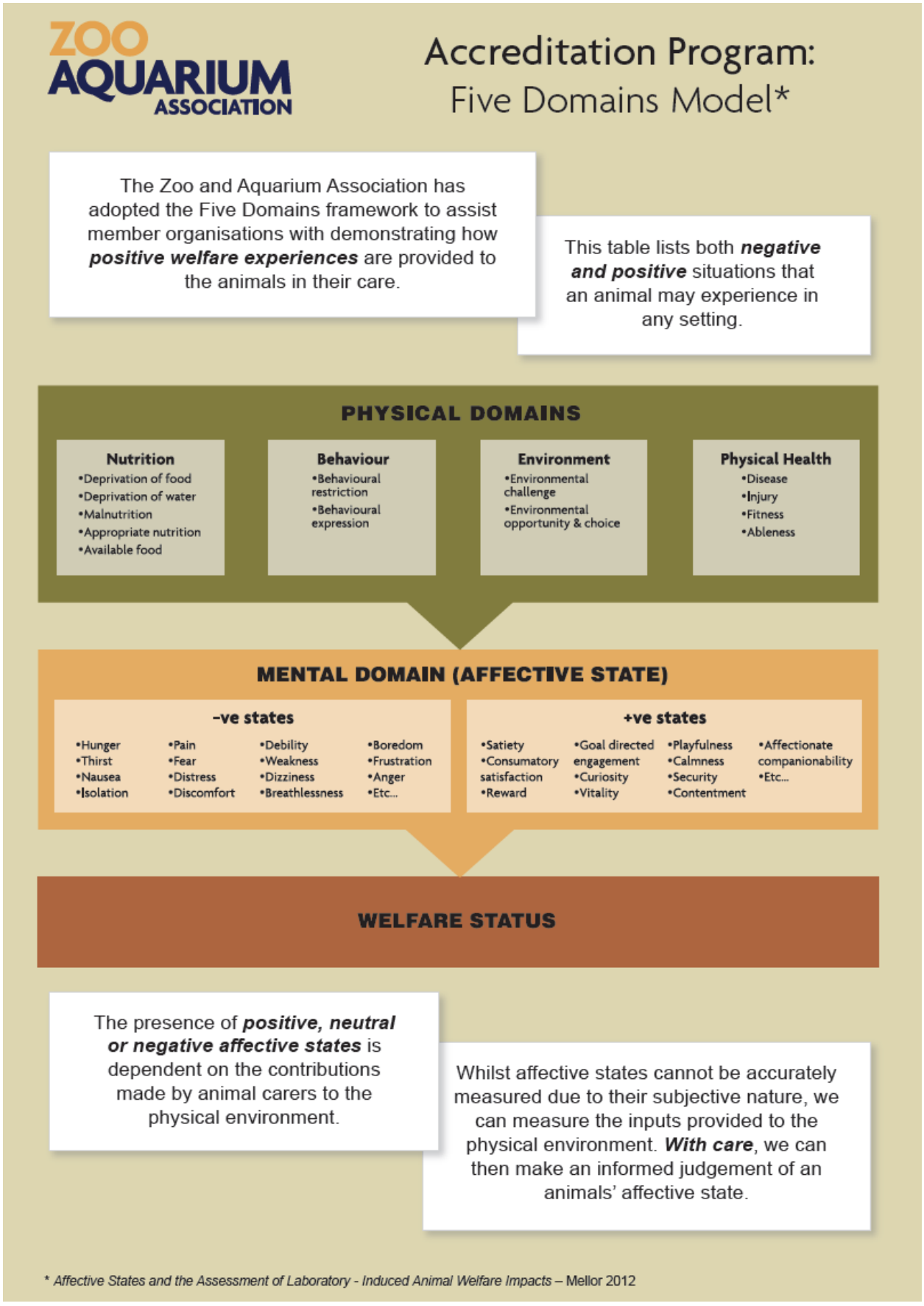
Recently our Association has adopted the Five Domains of Animal Welfare developed by Professor David Mellor and colleagues from Massey University in our ZAA Animal Welfare Position Statement. The Five Domains takes animal welfare beyond the Five Freedoms – the Five Domains model assesses not only the physical well-being of the animal but also its emotional and mental (affective) states.

The Five Domains also form the basis of the new Accreditation programme for ZAA members developed by our regional Accreditation and Animal Welfare Committee. This programme was launched in March 2014. All members will be assessed solely against animal welfare outcomes for their Accreditation. Other certification programmes such as Qualmark in New Zealand can be used to assess other areas in the zoo or aquarium organisation for certification purposes but animal welfare is a specialist area requiring more refined assessment by subject matter experts.

Many zoos across the world are now establishing Animal Welfare Committees with both internal and external members for transparency and to provide differing viewpoints. These Committees are seen as significant for those zoos that have established them and while the processes of the Committees may vary they have animal care at their heart.

The World Association of Zoos and Aquariums (WAZA) is currently developing an Animal Welfare Strategy based on the Five Domains model. This is being led by Susan Hunt, Chief Executive of the Perth Zoo and President Elect of WAZA.

Gone are the days of easy-to-clean, desolate zoo enclosures or, in fact, cages. Good, modern 21st century zoos think and behave differently. They contribute over US$350m to animal-related field conservation projects globally every year. They support


ZOO AQUARIUM ASSOCIATION
Accreditation Program:
Five Domains Model*
The Zoo and Aquarium Association has adopted the Five Domains framework to assist member organisations with demonstrating how positive welfare experiences are provided to the animals in their care.
This table lists both negative and positive situations that an animal may experience in any setting.
PHYSICAL DOMAINS
Nutrition
•Deprivation of food
•Deprivation of water
•Malnutrition
•Appropriate nutrition
•Available food
Behaviour
•Behavioural restriction
•Behavioural expression
Environment
•Environmental challenge
•Environmental opportunity & choice
Physical Health
•Disease
•Injury
•Fitness
•Ableness
MENTAL DOMAIN (AFFECTIVE STATE)
-ve states
•Hunger
•Thirst
•Nausea
•Isolation
•Pain
•Fear
•Distress
•Discomfort
•Debility
•Weakness
•Dizziness
•Breathlessness
•Boredom
•Frustration
•Anger
•Etc...
+ve states
•Satiety
•Consumatory satisfaction
•Reward
•Goal directed engagement
•Curiosity
•Vitality
•Playfulness
•Calmness
•Security
•Contentment
•Affectionate companionability
•Etc...
WELFARE STATUS
The presence of positive, neutral or negative affective states is dependent on the contributions made by animal carers to the physical environment.
Whilst affective states cannot be accurately measured due to their subjective nature, we can measure the inputs provided to the physical environment. With care, we can then make an informed judgement of an animals' affective state.
* Affective States and the Assessment of Laboratory - Induced Animal Welfare Impacts – Mellor 2012

animal welfare and take their responsibility to their animals very seriously. They engage their communities with positive actions for the environment. They work with partners to create a better planet for people and animals. Conservation of animals in the wild is at the heart of all they do.

Of course there is still what the zoo community and society at large would class as bad or substandard zoos around the world. These places are collections of poorly kept animals in unsuitable housing and with little compassion for their plight. These places do not meet the standard required although organisations like Wild Welfare and some world class zoos are working with some of these 'zoos' to improve their animal care. It is a daunting task and not the subject of this paper. That is another paper entirely.

This paper will also not discuss the role of zoos in advocacy – suffice to say that with over 700 million visitors worldwide and growing, zoos are well placed to tell conservation stories and reconnect disconnected urban people with nature in safe and engaging ways. The social science of conservation psychology and visitor engagement is a growing area of expertise and good zoos are embracing this role. Dr Nikki Harré from the University of Auckland talks about humans being happiness seekers and zoos seek to be happiness attractors. Conservation psychology is an increasingly growing area in the conservation sphere. Animal care plays a major role in setting the scene for these activities to occur with zoo visitors and communities – without well cared for animals the advocacy role of zoos would fall flat. However, the advocacy role of zoos and their ability to connect wild conservation stories through engaging zoo-based learning and creating a love of animals in the community cannot be dismissed or understated. Much new research and understanding is developing in this area globally and it is indeed changing the way modern zoos go about engaging visitors with both conservation and sustainability messages.

The subject of this paper is to explain how animal care has progressed in modern zoos and how it contributes to the mission and purpose of these zoos as the central tenet of their existence.

Zoos began many years ago as collections by kings and princes to show their status. The more exotic the animals, the more powerful the king must be. The first menagerie opened to the public was in Paris in 1793 during the revolution. This idea of collecting animals is a human phenomenon – we are curious about other creatures. This collecting mentality progressed to collections of curiosities and then to science-based collections for research. During the 1950s we were still in what we call 'postage stamp' collection mode – that is, zoo directors, often from a scientific background, were interested in collecting unusual animals often for their own interest. This started to change in the 1980s when curators became quite interested in the scientific potential of the collections of animals in their zoos. The animals became separate from the people who visited the zoo whereas previously to this there was quite close contact. Chimpanzee tea parties and elephant rides may seem abhorrent to us today but they were crucial in connecting people to animals at that time. We have continued to develop as a zoo community, like most organisations, where today zoos are conservation-based organisations with a real remit to connect people with nature, provide excellent animal care, support field conservation outcomes and create forums for discussion about improving the planet. Conservation is a relatively new area of science by comparison to zoo-based science of the past and we are now in the time where both field conservation and zoo-based conservation are parts of the same continuum to save species.

So where does animal care fit within this new phase of zoo growth and change?

For most zoos this is the main undertaking of their organisations. Without it the zoo is not making the grade and is subject to community criticism. Most zoos would not question that they have a responsibility to their animals to provide the best care available, be that veterinary care, husbandry practice, quality housing, and opportunities for natural behaviour and enrichment. Most modern zoos have qualified vets and veterinary nurses on staff, most keepers have degrees or masters qualifications and at the least have animal management certificates. Wellington Zoo partners with Massey University to build capability for New Zealand in the wildlife and zoo medicine field through the three-year Masters in Wildlife and Zoo Medicine. The qualified vets in this programme rotate on three-month placements at the Zoo and spend the other nine months at the university. Animal care in a good zoo is complex and requires critical thinking, good decision-making and empathy.

The ZAA Australasia has developed a new accreditation programme for members based on the

Five Domains of animal welfare model developed by Professor David Mellor and colleagues. In order for ZAA members to be accredited they must address all animal welfare concerns and show how they are providing best care for their animals.

In the Five Domains model, the four physical or functional domains (nutrition, environment, health and behaviour) are concerned with biological function, or physical well-being, whereas the fifth domain, the mental state, considers the 'affective state' or psychological well-being, and represents the animal's overall subjective feelings and experiences and hence this fifth domain is a key element of animal welfare. An animal may have positive or negative emotional states and it is the balance between these subjective experiences that can influence an individual animal's "Quality of Life" (Mellor 2013).

A positive affective state arising from the presence of positive experiences and sensations, with the avoidance of, or minimal, negative experiences, is therefore important to safeguard and ensure good animal welfare and this can be achieved when the physical (nutritional, environmental, health and behavioural) as well as psychological needs are addressed (Mellor 2013; Portas 2013). However, an individual's mental state and hence its welfare can vary from one point in time to the next as the different sensations it may experience during its lifetime change (Mellor 2013; Portas 2013). Thus, it is the complex interactions between each of the five domains that, in combination, may determine an animal's overall welfare status.

With this in mind, implementing management techniques and standards that promote positive physical and mental health for every species accommodated within zoological institutions, whilst also preventing unpleasant experiences for the animal, is fundamental to the care of wild animals in captivity. This can be accomplished by, for example, providing appropriate nutrition to meet the animal's biological needs which is presented in a manner to satisfy its feeding behavioural requirements, the provision of environmental choices, access to con-specifics (as appropriate) and access to a complex, variable and stimulating environment, in addition to the continued provision of high standards of both husbandry and veterinary care.

The following ZAA Accreditation Welfare Questionnaire is used to assess members during site visits:

The aim of the site visit is to validate the positive welfare outcomes found at our member institutions as part of Accreditation. Reviewing of physical evidence, like reviewing diet sheets and medical protocols, is not the aim of this. The aim is to validate the outcomes for animal. So the form of this will be a verbal discussion at the enclosure. The institution can nominate whoever they wish to be present. The visiting Accreditation officer will chat with whoever the institution wants to be present to gauge a level of understanding of the effects of any of the criteria on positive animal welfare. They will look at the animals, taking photos and / or videos showcasing each of the physical domains, to allow for impartial validation of their report by impartial members of the Accreditation and Animal Welfare Committee, who may have greater species specific understanding of the variety of species being assessed.

The visit looks at the physical domains that are described within the ZAA Animal Welfare Position Statement.

The Animal Welfare Position Statement follows the five domains model. At present only the four physical domains will be looked at. In the model it demonstrates that if the four physical domains are in a positive state then the fifth, the affective state, will also be in a positive state.

During the visit discussions are had around each of the headings below. The information following a heading gives examples of ways an institution can provide for the positive welfare of their animals. This list is not meant to be exhaustive, but gives ways an institution can demonstrate their understanding of positive welfare and what can assist in a positive outcome for their animals. However this list will be used for guidance for both the institution and Accreditation officer. These are all examples and there will always be an exception to every rule, be it at a species, individual or institutional level. An institution is welcome to show how their methods around any of the domains give a positive outcome in a differing way to those below. These will be documented and given equal weight to the examples below. An example of this may be a snake which has a limited diet of whole rodents. This is all that the species eats therefore there can be little variation in the diet. In this case variation would not improve welfare, and in fact may decrease it by reducing the consumption by the individual.

To give both transparency and robustness to the report photographs and videos of animals and their enclosures will be taken. The photos and videos will include examples of behaviours, social interactions, diet and its consumption, body condition, gait, and the enclosure. These will be supplied with the report to both the institution and the Accreditation and Animal Welfare Committee. The discussion will be recorded. This is only so that the Accreditation officer does not have to have the discussion whilst both writing and taking videos and photos. A copy of the recording will be available to the institution if required.

1. NUTRITIONAL DOMAIN

Diet provides for behavioural and nutritional needs of the individual animals

At different stages in its life an individual animals dietary requirements might change. A diet needs to take the varying stages of life and behavioural needs into account.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Fully for all ages and reproductive statuses	A proportion of the behavioural requirements as well as all of the nutritional requirements of the animals are met (eg a scatter feed but also food fed out in a single bowl all done once per day)	Does not provide for the nutritional or behavioural requirements of the animal	Body and coat condition Diet presentation Variety, quality and quantity of food Food preparation, storage and delivery Visitor feeding of animals

How is this achieved?	
How does this improve the welfare for the animals?	

Diet contains variety
Animals presented with variation in diet are more likely to experience positive states such as novelty, seeking, etc whereas animals who do not experience variety are likely to experience negative states such boredom, reluctance etc.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Yes - Regularly	Yes - sometimes	No	Variety of food given
How is this achieved?			
How does this improve the welfare for the animals?			

Food intake is monitored
If the nutritional intake is monitored and recorded it will allow for proactive management of the dietary needs of the individual animals within a collection. General body condition should be used as a measure of adequate intake.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Proactive management of nutritional matters is undertaken, by all necessary staff, rather than waiting for an issue to arise	Issues monitored Reports are reviewed by keepers and or vets as required	No or limited monitoring Limited vet/management involvement No knowledge of how an issue can be reacted to can be demonstrated	Demonstrated staff awareness
How is this achieved?			
How does this improve the welfare for the animals?			

Diet sheet is reviewed
Regular reviews of diet sheets increases likelihood that the diet will reflect contemporary understanding of a species dietary requirements, and as such are more likely to achieve positive states. A regular review of a diet sheet does not necessarily mean it is an adequate or inadequate diet. A diet might be able to be provided that mimics exactly what is eaten in the wild by that species, an example could be a Koala, without the need for regular reviews. Diet reviews that involve a variety of specialist resources can increase the likelihood that it meets behavioural as well as nutritional requirements. The resources could include Vets, Zootrition, keepers, curators and species coordinators. This is not an exhaustive list of options that could be utilised.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Diet sheet is reviewed at least every 12 months or prior to new individuals arriving in the collection or can be shown to meet the exact nutritional requirements of the species and individual without the need for regular review Multiple specialists, and resources, have been utilised that cover a whole range of species and nutritional knowledge when considering the makeup of the diet	Diet sheet is reviewed at least every 2 years or in reaction to an issue arising around groups changing, eg new arrivals that don't eat the existing diet, gravid females, infants or elderly animals being present in the group A small number of specialists, and resources, have been utilised that have some knowledge of the species and its nutritional requirements	Diet sheet is infrequently or not reviewed No specialists or specialised resources have been used when considering the makeup of the diet of the diet	Demonstrated staff awareness
How is this achieved?			
How does this improve the welfare for the animals?			

Timing, accessibility and presentation of the diet
A large proportion of species will benefit from having a number of feeds per day. This will increase the foraging time spent and can assist zoo animals to have a more natural activity budget. For social species it may improve the welfare of individuals within a group to feed in multiple places, allowing the subordinates to have full access to the diet.

There are some species, like large carnivores, where multiple feeds every day is not appropriate. Some of these may even benefit from a single large food item rather than lots of small ones. An example of this is African Wild Dogs where the social system is reinforced around who gets to feed on a carcass and in what order. Multiple feeding is not an appropriate measure for those species.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Multiple feeds each day, all at varying times and in a number of places within the enclosure. The diet is presented in such a way to give all animals within the social group access to the full range of the diet	2 feeds per day in 2 separate places within the enclosure	A single feed per day in a single spot of the enclosure	Demonstrated staff awareness Placement of food within enclosure
How is this achieved? How does this improve the welfare for the animals?			

2. BEHAVIOUR DOMAIN

Individual animals display species appropriate behaviours in their use of the enclosure, dietary preference, and in their interactions with any conspecifics within the enclosure or its immediate surroundings.

Species appropriate behaviours will demonstrate a positive outcome not only for the individual but also for the others that share the enclosure. Species appropriate behaviours can be specific to an individual rather than generically for the species it belongs to. An example of when this can be taken into account would be for animals that take part in interactive programs that display appropriate behaviours for their situation but that may differ from species normal behaviours.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Species appropriate behaviours are demonstrated at all times. This can include competition for mates and resources as long as it does not cause serious harm or distress to another animal within the enclosure or nearby	Predominately species appropriate behaviours are demonstrated. Any behaviours that are not appropriate for the species have no negative effect on the animals or on others within the enclosure	Few species appropriate behaviours are demonstrated. Inappropriate behaviours for the species that demonstrate a negative effect on the animal or other enclosure inhabitants are demonstrated. Note these behaviours would need to cause serious physical harm or distress rather than stress	Demonstrated staff awareness of species biology and life history A range of species appropriate behaviours observed
How is this achieved?			
How does this improve the welfare for the animals?			

Species appropriate social setting
Animal(s) kept in a species appropriate social setting are more likely to achieve positive states such as affectionate companionship, security etc. It should be noted that by being in a social situation there will always be an individual who is of the lowest social standing. This is both normal and natural, as well as essential to any healthy social structure. However just because an animal is of the lowest social standing it should not be allowed to suffer. It must be better off being part of the social group than being housed singly. Whereas species housed in inappropriate social settings will likely result in aggression, isolation, fear etc.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Yes with successful breeding and rearing of offspring occurring within the group	Non breeding	No	Witnessing the social structure and a range of interactions within a group Similar aged conspecifics growing up at the same time Mate choice where species appropriate Courtship, mating and rearing of offspring taking place
How is this achieved?			
How does this improve the welfare for the animals?			

Training and conditioning programs (if in use)
Training and conditioning can provide for a positive welfare outcome for the animals. This can lead to a number of outcomes, including getting an individual to display more species appropriate behaviours. One seen more often is that of medical conditioning, reducing unnecessary stress. Another example is training of animals to take part in interactive displays. This allows the animals to have greater contact without compromising the welfare of the animals or the safety of staff and visitors.

Note: the lack of a training and conditioning program in itself is not indicative of a neutral or negative welfare state but it is a tool that can be used to improve welfare if needed.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Training and conditioning programs take into account individual animal situations and improving the welfare of those animals	Training and conditioning programs take place to minimise unnecessary stress	No training or conditioning programs take place when it can be demonstrated that one could improve a negative welfare situation Training takes place that has a negative impact on the animal	Demonstrated staff awareness of the positive outcomes for the animal of the training and conditioning
How is this achieved?			
How does this improve the welfare for the animals?			

Species knowledge
Applied species specific knowledge is more likely to promote positive states as husbandry is better aligned with the species needs.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
A good understanding of species normal and natural behaviours can enable an institution to proactively manage the positive welfare of their animals	Some understanding of either natural (so wild behaviours) or normal (for zoo animals) behavioural repertoires can help to prevent negative welfare concerns	Limited species knowledge can lead to misunderstanding of behavioural indicators, which could lead to reduced animal welfare	Demonstrated staff awareness of species biology and life history
How is this achieved?			
How does this improve the welfare for the animals?			

Knowledge of the individual animal
In addition to species knowledge individual knowledge will allow the zoo to ensure they are providing suitable stimuli to enable the animal to demonstrate appropriate behaviours indicating a positive welfare state.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Can identify all individuals within an enclosure and understands each animals specific behavioural traits. This individual knowledge will help to understand an individuals social standing in the hierarchy and in turn help an institution to predict implications for actions to improve an animal's welfare.	Can identify individuals within an enclosure and has species knowledge but has little or no knowledge of specific behavioural history of the individuals	Cannot identify individuals within an enclosure that could to a lack of understanding of the impacts on individuals of their social standing and thus the impacts of any intervention by staff to improve welfare would not be known	Demonstrated staff awareness of individuals and their social status or the behaviour of the group for those that are impossible to identify individually

In some species an individual may be replaced by knowledge of a shoal, swarm or group. Examples of this may be for some fish, invertebrate species, flock of birds or any other large groups of highly social species			
How is this achieved?			
How does this improve the welfare for the animals?			

3. ENVIRONMENT DOMAIN

Environment supports behavioural needs
A species appropriate environment will provide for the basic needs of an animal. Eg a Penguin should have access to an adequate body of land as well as a suitable body of water that meets both the requirements it has on land and in the water.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
The environment provides for the physical and behavioural needs of the animals This can include a choice of 'micro-climates' – sun, shade, extra heat, near visitors, ability to get away from each other, visual barriers, sound barriers and a dynamic environment	The environment provides for the physical needs and some of the behavioural needs of the animal	The environment does not provide for the physical or behavioural needs of the animal	Demonstrated staff awareness of the environmental needs of the species The enclosure can be seen to meet the physical needs of the species and is fully utilised The exhibit can be seen to be well maintained The ability to manage the varying requirements of a social group
How is this achieved?			
How does this improve the welfare for the animals?			

Species appropriate climate / microclimate
Species appropriate climates and microclimates can have a profound effect on positive animal welfare. A good example would be a reptile enclosure that is warmer at one end than the other. This allows the animal to thermoregulate. Also due to differing levels of heat one end may have increased humidity at substrate level than the other.

Another example may be the use of a heated house for a mammal species. When it wants to the animal may enter the heated building

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
The animals within an enclosure have use of a variety of species specific microclimates within the enclosure and this is adjusted based on use/group dynamics and seasons	Ambient temperature and humidity requirements are met for the animals within the enclosure but little or no variation is available Eg animals have the conditions that are conducive to moulting etc	Correct climates / microclimates are not provided within the enclosure for the species held Eg are they shivering, showing signs of heat stress, etc	Demonstrated staff awareness of species requirements and their natural climates as well of that of individuals within a zoo setting Observed choices of microclimates that the animals can use
How is this achieved?			
How does this improve the welfare for the animals?			

Behavioural and environmental enrichment
An enriched environment will give an animal necessary stimulus. These stimuli should lead to positive behavioural indicators being demonstrated. Behavioural and environmental enrichment can include a wide variety of items including, randomly introduced and regularly changed objects, elements of weather, substrate changes, conspecifics within the enclosure and things that go on outside the enclosure that can be seen from within. There is almost no limit to the variety of ways an environment can be enriched. Should be species appropriate – objective should be to promote species appropriate behaviours and species appropriate activity budgets.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Enrichment happens randomly, for varying lengths of time on an ongoing basis. It is recorded to ensure it is not repeated on a regular basis at a regular time. Enrichment is evaluated which then informs future enrichment	Enrichment happens at a set time. The consequences of repetition are not managed making the enrichment routine rather than novel	Enrichment is not provided or is not species appropriate	Enrichment must provide for the capacity of the animals and to promote species appropriate behavioural opportunities
How is this achieved?			
How does this improve the welfare for the animals?			

Species appropriate substrates provided
Often a variety of substrates can have a positive outcome on a species environment. An example would be for some Parrot species that spend a percentage of their time on the ground exploring. A selection of varying substrates can increase the positive interaction between the bird and its environment. Another example could be the use of rocks and sand for some reptile species. Whilst the sand may be a suitable substrate that moves and constantly changes the rocks can be areas that are heated giving suitable basking spots. A positive welfare state might be indicated by sound foot condition, low parasite load. Animals that dig or burrow will require a different type of substrate to those that do neither.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Animals have access to a variety of appropriately placed suitable substrates are provided within the enclosure	A single suitable substrate is provided within the enclosure when more could benefit the welfare of the animals held within the enclosure	There is a lack of any suitable substrates within the enclosure	There is a variety of substrates which the animals do use Demonstrated understanding of the animals use of the varying substrates
How is this achieved? How does this improve the welfare for the animals?			

Appropriate shelter and cover provided

Species, especially those held outside, require shelter and cover from the elements. Even those held within indoor enclosures should have the choice to move out of view if they desire. Is there capacity for animals to control shelter/cover, eg close/open door, shower with warm/cool water, be in the sun or not, be in the wind or not. Are animals confined to dens for prolonged periods, especially if the species is active at night? Cover and shelter can be either man made or natural, or a combination of both.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Multiple species appropriate shelters and/or cover is provided which enables the animal/s to retreat to when it wishes	Appropriate shelter is provided, but there are times when the animal might not be able to access this, without causing negative welfare	Inappropriate or no shelter and little or no cover is available for the animal to retreat to or the animal has no ability to make use of provided cover	Species appropriate shelter and cover observed Demonstrated understanding of the species requirements
How is this achieved? How does this improve the welfare for the animals?			

Species appropriate furnishings

Substrates are not the only part of an enclosure that an animal will interact with. Furnishings like plants and trees, rock mounds, branches water bodies are all part of the environment the animal will interact with. Varying ages and sexes of animals within an enclosure may influence the furnishings required, for example aviary perching may need to be of different diameters to accommodate differing age birds.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Complex variety of furnishings are provided to promote a wide range of species appropriate behaviours	Limited variety of species appropriate furnishings are provided	Furnishings do not meet the needs of the species or individuals within the enclosure	A variety of furnishings observed that the animals can use to provide for positive health and behaviour
How is this achieved?			
How does this improve the welfare for the animals?			

4. PHYSICAL HEALTH DOMAIN

Proactive health care

Animals are proactively monitored to ensure that any health issues are prevented where possible or are detected before they become an issue for the animal. Feathers, coat and body condition may all be indicators of positive proactive health care as well as regularly maintained environments.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Regular health screening takes place and is managed under the supervision of a vet. The level of health management takes into account individual animals' needs. There is a requirement for limited reactionary health care. Resources are always available for any care required	Regular health screening schedule with vet consultation. A greater proportion of health care is reactionary. Resources are available for any care required	No proactive health screening takes place. All health care is reactionary Resources may not be available to provide for the health care of the animals	Demonstrated understanding of the proactive health care that takes place and zoonotic disease for the animals A regularly maintained environment is observed Good body and coat condition is observed The animals appear to be healthy
How is this achieved?			
How does this improve the welfare for the animals?			

Individual and group care, including aged and health compromised animals

Aged animals often develop severe medical conditions. Some animals will have congenital or longer term issues. Proactive monitoring of not only their health but overall welfare is essential to ensure they remain in a positive welfare state.

Positive Welfare State	Neutral Welfare State	Negative Welfare State	Supporting evidence
Animals, including those that are aged or compromised, routinely have their medical and welfare condition assessed by keepers and veterinarians	Animals have routine health screening	No proactive health or welfare monitoring takes place	Demonstrated understanding of health monitoring regimes for all the animals in the enclosure
How is this achieved?			
How does this improve the welfare for the animals?			

So, good zoos have come a long way and the journey is not over. The more we know, the more change is required. Caring for animals in the 21st century is a complex undertaking. As we discover more and accept that animals have feelings and emotional needs as much as physical ones then this area of zoos will change. Zoo management is a complex beast and animal care is at the forefront of that complexity. Zoo carers will and should become more expert in how they care for their animals and they will be continually improving in this area.

Without this attitude to animal care, zoos are open to criticism from society and, rightly so, in the 21st century.

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The blind leading the blind: animal facility staff and researchers working together to reduce bias in animal research

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Introduction

Anyone who watches the news frequently might be aware of a very wide and deep *fissure* that exists in science today. They will be aware of this *fissure* because every week it appears that a disease is cured by a new compound. This compound might one week be found on the skin of a frog that lives deep in the Amazonian rain forest, and then the next week perhaps it was extracted from an everyday food like dark chocolate or red wine. This astounding news article would hardly cause a modest arousal to a seasoned news watcher, as they would be acutely aware that these discoveries happen every week, yet for some reason the diseases mentioned continue to flourish unabated. This phenomenon is caused by the aforementioned deep *fissure* in science that divides preclinical and clinical research. The news article would refer to new research that has shown outstanding effects in an animal model of disease or perhaps cells grown in a petri dish (preclinical research). However, these outstanding effects almost never correspond to *any* therapeutic effect when investigated in clinical research on the human condition, and with every failure of these drugs to work in the *Homo sapiens*' version of the disease the *fissure* widens. In science circles this *fissure* is referred to as a *translational failure* and the conservative estimate for the ratio of drugs that make it across the *fissure,* to those that fall into its abyss, is approximately *one* in every *three hundred* (Mak et al. 2014; Thomas et al. 2014). There are probably many factors that are causing the translation failure rate to be so exceptionally high, but fortunately there are scientists that are investigating why preclinical science is failing. And what these investigations have uncovered is that although the scientific method has come a long way, we have far from perfected it. Perhaps minor methodological improvements could build a bridge over the *fissure*, or at least reduce the gap enough to improve the translational failure rate to a more acceptable level.

The evolution of the scientific method

For most of recorded history those who sought medical attention had a greater chance of dying than those who stayed at home (Fara 2009). Hospitals were putrid pits of disease at which the mostly well intentioned doctors administered treatments that were typically ineffective or harmful (Fara 2009). Leading medical physicians would prescribe mercury pills to induce vomiting, hysterectomies to reduce female hysteria (the word hysteria comes from the Latin word for uterus – *hystera*) or perhaps a dose of gold to cure jaundice; since jaundice causes the patient to turn gold surely gold would cure jaundice (Burgh 2009). These treatment techniques seem so unlikely to work that it is easy to think that these men were in some way unintelligent. However, for the most part this is not the case; these were brilliantly intellectual people that used their best judgment to care for the patient.

So what has changed? Well, many people would say that healthcare improved with each new discovery: aseptic techniques; penicillin; chemotherapy; statins, etc. We simply added to the pile of knowledge and

this resulted in more treatments and better healthcare. However, I would argue this misses the underlying processes of discovery and that the advancement of modern medicine should, instead, be attributed to the development and refinement of *science* itself. For example, a basic scientific approach to medicine would be collecting a group of people who are at the same stage of a particular disease and then breaking them up into smaller groups and giving each group a different treatment to assess which treatment works best. This seemingly fundamental concept of medical research became widely accepted only after it was reported by a Royal Navy surgeon named James Lind in 1747 (Singh & Ernst 2008; Burch 2009). At the time scurvy was reported to kill more naval sailors than armed conflict and as scurvy began to develop amongst the sailors of the *Salisbury* vessel, Lind collected the sufferers and split them up into groups of two which he matched for disease severity (Singh & Ernst 2008; Burch 2009). Then he gave each group a different treatment, all of which were based on his erroneous theory that acid should help the terrible condition (Singh & Ernst 2008; Burch 2009). Fortunately, one group was given citrus acid in the form of limes, which contain the only treatment for scurvy – vitamin C (Singh & Ernst 2008; Burch 2009). This group was on a miraculous path to recovery, one even returned to work, until they ran out of limes. From this study Lind concluded that a citrus syrup should be taken on future vessels (Singh & Ernst 2008; Burch 2009). Unfortunately, the process of making the syrup involved boiling which greatly reduces the levels of active vitamin C (Singh & Ernst 2008; Burch 2009). So although Lind had seemingly missed the key treatment of scurvy, he was the first to describe the fundamentals of a clinical trial.

So it is wrong to say that medicine has improved as we discovered new and better therapies, because for most of recorded history the very techniques of investigation were not refined enough to make these discoveries. We simply could not have developed statins (a cholesterol-lowering drug) to treat the development of heart disease before the scientific method was refined enough to detect the unobvious impacts of the drug. Of all the refinements to the scientific method, randomisation and blinding have been particularly important to the development of new therapies for disease. Yet despite the history of these techniques, which clearly illustrates their importance to the scientific method, they are not commonly used in preclinical research (Sena et al. 2014).

One problem clinical research faces is how to divide patients into the various treatment groups. Historically, the allocation would be performed by the researcher in a *subjective* manner. Which begs the questions, did the researcher, subconsciously or consciously, place the sicker patients in one group and only give the treatment they believed should work to the patients that were likely to survive anyway? This subjective and undefined method of treatment allocation was a breeding ground for potential biases. What was needed was an *objective* method that divides the patients as opposed to a *subjective* method.

Randomisation is the best example of an objective method and could be as simple as flipping a coin to determine whether a patient receives treatment A or treatment B. One of the earliest reported clinical trials that used an *objective* method for treatment allocation was by the young Scottish surgeon Dr Hamilton in 1809 (Singh & Ernst 2008). Dr Hamilton and his colleague Dr Anderson believed that bloodletting was not an effective treatment for any ailment, while their older unnamed colleague believed, as most doctors did at the time, draining between 500 ml and 2.5 L of blood from a patient is an effective treatment for many ailments such as fever or inflammation (Singh & Ernst 2008). As patients came into the clinic they were systematically allocated to be treated by Dr Hamilton, Dr. Anderson or the unnamed doctor. It was discussed that the treatments should be standard between all doctors except Dr Hamilton and Dr Anderson would not perform bloodletting (Singh & Ernst 2008). After each doctor had seen 122 patients, the survival rates were compared. Dr Hamilton had lost four and Dr Anderson had lost two, which were very good results for doctors at the time. The senior doctor's success rate explains why this unnamed doctor was unnamed, for he had lost 35 of his 122 patients (Singh & Ernst 2008). And with that, there was now robust evidence against a procedure that had been utilised by doctors since before Hippocrates stated that doctors should "first do no harm" in the 5th century BC. This demonstrates the importance of the development and refinement of science. Bloodletting, a medical practice that was performed and observed for over 2000 years, was detrimental

to the patients that received the treatment, often to a lethal degree. Yet not until the scientific method had been refined could this grossly pathological practice be seen for what it was and removed from use. However, due to doctors not willing to acknowledge that their profession had been killing people, Dr Hamilton's research was largely ignored causing bloodletting to last another century until it finally faded from medical practice (Fara 2009).

Another crucial advancement in experimentation was the notion of blinding. This is where the assessor and administrator of a treatment is unaware what treatment is being given. Blinding was largely devised to account for the *placebo* effect. This effect is where the patient benefits merely because they believe they are receiving an effective treatment and it is caused by both a change in the physiology of the patient and a change in the perception of the ailment. The first description of the placebo effect has a very interesting history. Any new discovery is met with theories about human health. After X-ray machines were first invented theories were proposed that exposure to X-rays invigorated the body and those who could afford it may delight in an energising daily X-ray (Sansare et al. 2011). Cell phone towers on the other hand, were initially thought to cause various diseases, including cancer and migraines, and erecting a tower near a school or kindergarten was often met with public protest (Dolan & Rowley 2009).

Nowadays, X-rays are well known to cause cancer and the only response to the erection of a new cell phone tower is gratitude for the faster Facebook updates. So in the 1780s, when Galvani Lugi used twitching frog legs to suggest that the body uses electrical fluid to activate muscle activity, it is not surprising that some entrepreneurial fellow decided that this electrical fluid must be involved in human health (Singh & Ernst 2008). The American physician Elisha Perkins proposed that noxious electrical fluid must build up in painful and inflamed areas (Singh & Ernst 2008). He developed two metal rods made from exotic materials which he named tractors; these tractors could be passed over the problematic area and would drain it of the agitating electrical evil (Singh & Ernst 2008). Due to the exotic material the rods *must* be made from, the cost of this equipment was 5 guineas, which at the time was around half the annual wage of a labourer (Singh & Ernst 2008). This business was very profitable until a skeptical and frugal British physician by the name of John Haygarth decided to investigate cheaper alternative metals that could be used to make the Perkin's tractors (Haygarth 1800; Singh & Ernst 2008). As Haygarth researched different materials he found something very odd, not only did cheap metals work as well as the exotic originals (which were actually made of the relatively cheap metals brass and steel), but non-conducting materials like wood had an equivalent therapeutic effect; in fact anything he waved over the inflicted area with convincing conviction for the required 20 minutes appeared to alleviate the patients' symptoms (Haygarth 1800). Haygarth was fascinated *"*to a degree which has never been suspected, what powerful influence upon diseases is produced by mere imagination*"* (Haygarth 1800; Singh & Ernst 2008). He also noted that this probably explains why treatments worked better in the hands of more famous and expensive physicians. This was a major discovery for the scientific method; the simple comparison of the patient before and after treatment had a fundamental flaw. The patients' mere imagination would corrupt the results, meaning any before and after comparison was quite likely to be completely erroneous. The problem of the patients' imagination could be solved by comparing any treatment to a dummy treatment (placebo), just like Haygarth's wooden tractors. However, this practice did not become widely used until 150 years after Haygarth and his wooden tractors (Singh & Ernst 2008).

Interestingly, the placebo effect can work not only on the patient but also on the physician or the scientist as well. One famous example of this was the discovery that homeopathy works at the cellular level. Now to those who are unaware of the details of homeopathy this claim might seem quite normal, but to most scientists homeopathy is an amusing way to teach undergraduate students about dilutions. The principles of homeopathy state that a substance which causes symptoms will cure those symptoms in *extreme* dilutions; so caffeine keeps you awake and therefore will cure insomnia when diluted suitably (Singh & Ernst 2008). Now a normal dilution in homeopathy would be in homeopathic jargon a 30C dilution. C is the Roman numeral for 100 and so 30C indicates the solution is diluted 1 in 100, thirty times. On top of this extreme dilution, the normal dose given to a patient is one hundredth of a millilitre, which is less than one drop. To really explain what this means,

imagine taking half a teaspoon of pure caffeine, dissolving it in a ball of water the size of our solar system (using Pluto's orbit), then taking less than one drop from the ball of water and administering it for the treatment of insomnia (Singh & Ernst 2008). The patient would be more likely to win lotto three times in a row than receive a single molecule of caffeine in their treatment.

If not for the fact that in 2013 the homeopathic industry's estimated worth was $6.4 billion in the United States alone, homeopathy would be quite a humorous subject to most scientists (Singh & Ernst 2008). However, in 1988 a paper was published by Dr Benveniste and his laboratory group in the prestigious journal *Nature* which provided inexplicable evidence for homeopathy (Dayenas et al. 1988). The subjects of the paper were not patients reporting on subjective feelings or overall well-being, but cells under a microscope. The diluted solution used in Dr Benveniste's study was effective at eliciting a response in the cells at a 120C dilution, which is more than 1 billion billion billion billion billion billion times more diluted than the solar system caffeine example given above (Dayenas et al. 1988). *Nature* published the paper; however, as no phenomenon in science could explain the reported results, scientific observers selected by the *Nature* journal went to the laboratory that produced the paper to observe the experiment for themselves (Dayenas et al. 1988; Singh & Ernst 2008). The experiment involved applying a stimulant solution to the cells and observing the cells to see if they 'degranulate', which is a process where the cells eject their signaling molecules reservoirs. Dr Benveniste's laboratory group repeated the experiment for the observers and they got the same result; however, the person looking down the microscope at the cells and counting the number of degranulated cells was aware of what solution had been applied (Singh & Ernst 2008). The *Nature* observers asked them to repeat the experiment whilst blinded to cells which had been given normal saline and cells which had been given the extreme dilutions of the stimulant (which, given the dilution, was by all probability also normal saline) (Singh & Ernst 2008). Once blinded the effect disappeared, showing that it was the scientist observing the cells that was affected by the treatment and not the cells themselves. This isn't to say that Dr Benveniste and his laboratory were actively skewing the results; perhaps just minor differences in their interpretation of the cells degranulated state caused an unintentional and detectable level of bias. After the *Nature* journal reviewers published their observations, all scientists could then take a sigh of relief as this inexplicable result was now explicable. But, should they breathe a sigh of relief given that a vast majority of all preclinical scientific experiments at that time were performed in an unblinded manner? What other scientific 'discoveries' were actually just results of a subconscious change in interpretation or implementation of a method due to the all too human desire to produce interesting results which confirm the proposed hypothesis?

After Dr Hamilton's research clearly showing bloodletting was killing patients the technique took around 100 years to disappear from common practice. Similarly, the use of a placebo control only became common 150 years after the work of Dr Haygarth on the Perkin's tractors, as now again research techniques are in a period of delay. Dr Benveniste's work clearly shows that preclinical scientists must be blinded to the treatment groups and Dr Hamilton's research shows the unarguable robustness of randomised research and yet the practices of blinding and randomisation have not become common practice in preclinical research. In 2014, an analysis of preclinical animal research in the field of treating diseases of the brain found that only 20% of the research reported blinding of the assessor of the brain damage and 15% reported randomly allocating the animals into treatment groups (Sena et al. 2014). The percentage of research that is done in what many would describe as a rigorous manner appears dismal; however, medicine is improving; new drugs, new therapies, new diagnostic techniques and new surgeries are being developed every year. This begs the question, are these biases actually affecting research output? Do we need to change? Isn't preclinical animal research working anyway?

Does preclinical animal research work?

Animal research is incredibly useful and certainly does work. Surgical techniques are developed and practised on animals, which are then used in the clinic with huge translational success. Toxicology research in animals is very good at predicting which compounds are toxic to humans; generally compounds which are safe at high

doses in several mammalian families are very likely to be safe for humans. But the topic of this article is drug development, so does preclinical animal research work for drug development? To answer this question the definition of 'work' must be established and to do that we must look at the aim of animal models of disease. Preclinical research investigating novel treatments of disease induces pathologies in animals using a range of techniques. As the human disease cannot be replicated exactly, the aim is to produce a model that replicates the real condition as close as possible with the overall goal of *accurately predicting if a new therapy will work in the human disease*. Therefore, one way of answering the question "does preclinical animal research work?" is to answer the question "does preclinical animal research accurately predict if a new therapy will work in the human disease?" To this question we have some answers.

Let us look at the disease with the most preclinical research – cancer. Animal research in cancer normally involves growing cancer cells in a petri dish and then inserting the cells under the skin of a mouse and observing the growth rates of the cells. Then a treatment that had been shown to kill cancer cells in a petri dish experiment would now be given to the mice at different doses to assess if the drug can get to the cancer cells and kill them in the new setting of an animal. Now imagine a laboratory that is very successful and discovers 500 new compounds that appear to be effective in treating a range of cancers in these mouse models. Other laboratories will read about the compounds and perform similar experiments and report their results. The reported evidence will build until a pharmaceutical company (or public entity) deems the evidence to be worthy of investing. The company will fund a phase 1 clinical trial. Of the 500 new compounds originally discovered only 150 will make it to this stage of research (Mak et al. 2014; Thomas et al. 2014). A phase 1 clinical trial will assess what the drug does in healthy males: Where does it go? What does it do? What does it turn into? How fast is it excreted? Based on these results the company may decide to go to a phase 2 trial, which is a moderate sized trial on sufferers of the disease. Less than 13 of the original 500 compounds will make it on to this stage (Mak et al. 2014; Thomas et al. 2014). The drugs that appear to have some therapeutic effects will go on to a large-scale phase 3 trial of disease sufferers. Of the 13 drugs in phase 2 trials, 5 will be tested in phase 3 trials and of these maybe 1 or 2 will receive food and drug administration (FDA) approval and be used in the clinic (Mak et al. 2014; Thomas et al. 2014). So from 500 original drugs that were therapeutic in preclinical animal models, 1 or 2 are found to be therapeutic in the clinic (Mak et al. 2014; Thomas et al. 2014). From this it seems that animal models fail to accurately predict what occurs in the clinic. Keep in mind the fact that this failure rate from this example is what you get when you boil the effects of the drugs down to binary data, *therapeutic or not*; when the effect sizes are compared the predictive power of the animal model drops even further. Even if a drug is found to be successful in the clinic setting, it is often far less effective than the results reported in the animal studies.

From this it appears the preclinical animals models are doing a poor job of predicting what would happen in the human condition but is this because of blinding and randomisation? What about other obvious shortcomings of animal research such as using mice, which are obviously not human? Mice are smaller and have different metabolisms to *Homo sapiens*. Is it not possible that the 300 failures for every 1 success is the expected failure rate given the biological differences between the species they are tested on? One answer to this is that in mice: caffeine increases activity; methamphetamine is addictive; marijuana effects memory and appetite; Prosac™ reduces anxiety; antibiotics selectively kill bacteria and not the cells of the mouse; cocaine is addictive; nicotine is addictive; aspirin reduces pain and swelling; sunscreen prevents UV damage; agent orange causes deformities; and the list goes on. The number of drugs that have similar effects in humans and mice is much larger than those which act substantially differently. Another answer to what is causing the 300 to 1 failure rate lies in the history of an antioxidant drug named NXY059.

Case study: NXY059

According to the current paradigm of human physiology, our bodies are constantly producing free radicals (oxidants) which are highly reactive and potentially damaging to the cell. To counteract this, our cells are also producing antioxidants which safely react with free radicals diffusing their damaging properties. In healthy tissues, antioxidants and free radicals are in balance and both play an integral role

in normal physiology. However, in many disease states these become imbalanced and free radicals can reach high concentrations within the tissues. Free radicals are seen as dangerous molecules as they can react with components of the cell and cause them to malfunction; DNA can mutate, proteins can change shape and membranes can become leaky. The health and nutraceutical industry has caught on to this idea and that is why you will see "rich in antioxidants" everywhere as you peruse through the supermarket or pharmacy. Following a stroke, there is a huge rise in the concentration of free radicals within the brain and this was/is believed to contribute to the brain damage caused following a stroke. Therefore, preclinical research went into developing antioxidants that were potent and could penetrate into the brain to the site of injury; this led to the development of the very promising antioxidant NXY059 (Shuaib et al. 2007). Following the first neuroprotective animal study in 1999, animal research piled up with a vast majority of studies reporting dramatic therapeutic effects (Macleod et al. 2008). Some studies reported almost complete protection from brain damage in animal models of stroke (Mak et al. 2014; Thomas et al. 2014). A private company decided it was worth investing and designed a moderately sized phase 2 trial (Shuaib et al. 2007). This trial reported promising yet inconclusive results and so the company decided to pursue it further and organised another phase 2 trial with more patients (Shuaib et al. 2007). This time over 3000 subjects were enlisted in the study which became the largest clinical stroke trial in history (Shuaib et al. 2007). The company never released the true cost of the studies but it is estimated to have cost well over US $100 million.

When the results came in from the 362 centres from 31 different countries, there was no detectable effect of NXY059 on stroke (Shuaib et al. 2007). It was a resounding failure and the search for why it had failed began. Although there was probably more than one cause of this failure, some very clever preclinical researchers produced a meta-analysis (a study of studies), which clearly implicated failures of preclinical experimental technique resulting in NXY059 being found to be far more therapeutic than it may actually be (Macleod et al. 2008). McLeod and his colleagues' meta-analysis compared the reported effectiveness of NXY059 in multiple animal studies with what steps the studies used to reduce bias. What they found was very convincing; studies which reported the use of randomisation found the NXY059 was 2-fold *less* effective than studies which did *not* report randomisation (Macleod et al. 2008). Furthermore, producing a stroke in an animal requires surgery and what McLeod's study found was that if the surgeon was aware of which treatment the animal was going to receive, NXY059 was ~2-fold more effective than the studies where the surgeons were blinded to the treatment group (Macleod et al. 2008). Somehow the severity of the stroke was subconsciously manipulated by the surgeon!

This study went further and found that perhaps it is not that mice and rats are inappropriate animals to predict human conditions but that we are using the wrong mice and rats. A vast majority of rodent stroke studies use healthy young animals, when strokes normally occur in old and hypertensive humans (Ford 2008). The meta-analysis found that studies which used hypertensive animals reported NXY059 to be 2.2-fold *less* effective compared with studies that used young healthy animals (Macleod et al. 2008). Collectively, this meta-analysis showed that if animal studies had used randomisation, blinding the assessor of brain damage, blinding the surgeon of the treatment groups and old hypertensive rodents, NXY059 would have been found to be substantially less effective than what was previously reported (Macleod et al. 2008). This would have been considerably less appealing to the pharmaceutical company and perhaps the human trial would never have been done. Despite the failure of NXY059 and despite the beautiful work by McLeod and his colleagues, a recent meta-analysis found that there has been no increase in the use of randomisation, blinding or hypertensive animals since the NXY059 failure (Philip et al. 2009). Sadly, it seems that the lag between discovery and acceptances, which we saw with Dr Hamilton and Dr Haygarth's work, seems to be unavoidable, even in this information age we live in today.

Blinding – more complicated than you think

Most pharmacological animal research requires two steps which must be blinded. First, the induction of the disease must be blinded to what treatment the animals will receive and then after the treatment the scientist must be blinded again while assessing the severity

of the disease. The first blinding can occur through appropriate randomisation. First the animals are randomly assigned to either have the disease induced or not. Then simply randomly allocating the diseased animals to the treatment groups after the induction of the disease will blind the scientist performing the procedure to what treatment the animal is about to receive. This could be as easy as flipping a coin to see if they receive the drug or the placebo control. The next stage in blinding should be done after all the surgeries and drug treatments have been completed. This can be done by a colleague entering the animal room and replacing the animal information cards with a card with just a letter on it and recording which letter corresponds to which animal.

There are examples where things are not that simple and each situation must be worked through to develop an appropriate method. One of the most difficult situations is the use of genetically engineered (GE) mice which are visibly different to the normal control mice, such as the hairless GE mouse that is lacking the vitamin D receptor. How can you perform a surgery or an assessment blinded if merely looking at the mice unblinds you? Well this has a less simple solution and requires systems set up in university departments and private organisations to provide a solution. This could be that there are qualified staff in the animal care facilities who are capable of inducing the disease and giving the drugs without being aware of the proposed hypothesis. Bias can be reduced by acting independently and in a systematic fashion. Given the 2-fold increase in the effectiveness of NXY059 in animal studies that did not blind, it seems that it is not viable to simply say sometimes blinding is too hard.

We must at every stage try and reduce bias in research as biased results are simply unethical. If the results of the work cannot be applied clinically due to bias, which was at least a contributing factor in the NXY059 case, how can it be justified ethically? Biased research causes unnecessary animal suffering, unnecessary expenditures by governments and private organisations on preclinical and clinical research, unnecessary human experimentation, and it undermines the integrity of science. With continued failures of animal research, there is a risk it will lose public support and, with that, governmental support both financially and in the policies made. So what can be done?

What can we do about bias?

Scientific rigour has always been monitored by the trusted and respected "peer review system". This is where a study is written up and sent to a journal, then the journal editor sends this on to several experts in the field who voluntarily review the research. Documents are drawn up by the reviewers and are most often full of suggested new experiments and revisions, or the reviewers could outright reject the research due to poor design. Once the study is written up in a way that is acceptable to the reviewers it is published by the journal. This system is full of problems that would take at least a whole new article to address, so this article will only address one: money. The journals have to make money and they normally do this through selling subscriptions to read the research published in the journal. Therefore, universities have to pay huge subscription fees to ensure all good research that is published is available to its students and researchers. This also means that the general public will find it very hard to access journal articles, yet they will find it easy to access blogs and "information sites" on the internet (and we wonder why unfounded cures, fad diets, homeopathy, iridology, etc., persist in the age of modern science). This model essentially means that private journals appear to own knowledge and can sell it on the free market. But this also means that the journal is looking to have a quality *product* to ensure universities will purchase subscriptions. Because of this, the journal would rather reject low-quality research in order to maintain the standards of the journal.

Nevertheless, the fact that these journals essentially own and sell knowledge is seen by some as a crime against freedom of information. This perspective resulted in the proliferation of a new model – the open access journal. These journals still needed money to operate and so would charge the scientists for publishing their work in the journal (often around $3000). The journal would then allow the public to view the research for free. But the problem with this model is that there is less motive to reject low-quality research. As they are not selling subscriptions the *product* quality is not as important as *quantity* for the business to be profitable. There is no governing body controlling creation or operation of these journals and, as the journalist and biologist John Bohannon found, this combination of monetary motive and

science does not have good results. Bohannon organised a sting operation where he used a computer program to generate more than 250 papers which were completely fabricated, with made up authors from fictional universities (Bohannon 2013). The papers were designed to have fundamental flaws in them including a dose-dependent effect that was non-existent, no relevant controls and blatant failures in basic experimental design (Bohannon 2013). He submitted these papers to open access journals and was accepted and ready to publish in 157 of them and rejected by 98 journals (Bohannon 2013). Bohannon found that the open access journal model had generated a system where scientists could essentially pay to have their work published regardless of the quality of the research. It is clear from Bohannon's sting operation that scientific rigour cannot be left to the ungoverned "peer review" publication systems, so where can the standards be set?

There are two points before publication in which the experimental design can be assessed. The first point is the funding body. Most of science relies on research grants to fund their work. Applications are drawn up and submitted where they are reviewed by respected scientists and a few are selected to be funded. However, these applications are not full of experimental detail; this is because a scientist from one field would find it hard to review the methods of hundreds of applications from other fields of research when they are not familiar with the methods of that field. As a result minor details like blinding and randomisation are left out along with the technical details of the methodology. Research applications are about selling an idea, so they are filled with how important, novel and publishable the research would be, not the finicky details of methodology. So while new systems could be introduced to assess experimental rigour at this stage, it currently seems like something which would be seen as too hard.

The second review process all animal research must go through is the ethical approval process. Now first it should be stated that poor quality research has profound implications for the ethical use of animals. What justification can there be for the use of the animals that suffered in researching the effect of NXY059 on stroke? At last count there have been over 1300 compounds that have been found to be effective in animal models of stroke and yet only one of these compounds is currently used in the clinic. The quality and reliability of animal research is integral to it being ethical. Now unlike the research funding applications, the animal ethics applications are long and probing of the technical details of the methods. The Animal Ethic Committees (AEC) have to know every interaction with the animal to ensure it is ethical. Therefore, I would argue that the animal ethical review process is the best point in which the methods of blinding and randomisation can be assessed. It should be at this point that universities and research facilities can ensure that the research performed is of a high standard and therefore has the greatest applicability to human disease and consequently is the most ethical use of animals in research. Currently in New Zealand a human ethics application must be filed to perform a clinical study and this is reviewed by a committee. The human ethics committees require information on randomisation and blinding. Therefore, there is a working precedent of evaluating experimental rigour which could be followed in the animal ethics application and review process. I would argue that we owe it to the animals to consider this minor change in the review process.

Conclusion

The number of compounds which cross the *translational fissure* between preclinical and clinical success is dismal. Excellent research has illustrated that this translational failure is, at least in part, caused by a lack of experimental rigour in animal research. The basic steps of blinding and randomisation are not commonly performed despite historical and contemporary research that clearly demonstrates their importance. Part of the problem is the lack of regulation in the performance and reporting of scientific research. There is no governing body that is ensuring that the standards of preclinical research are such that the results are unlikely to be affected by biases. One point of regulation that has the ability and the desire to perform this regulatory role is the AEC. These committees must approve animal research and currently assess the importance of the research relative to the suffering the animals will experience during the study. Here I argue that scientific rigour is an ethical issue and should also be assessed by animal ethics committees. After all, how can animal suffering be justified if the results are of little relevance to the human condition?

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Behavioural management and enrichment: core concepts, and a little on mice

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No full paper was provided.

Abstract

The goal of this talk was to provide audience members with a rationale, arguments and examples to help with the implementation of enrichment and behavioural management in their home institutions.

The talk outlined the basic biological argument for the necessity of enrichment and good welfare to do good science. Simply put: animals exist to behave; behaviour exists to allow animals to control their environment and cope with physiological stressors that would otherwise be potentially deadly; animals which cannot exert control over their environment are teleologically, physiologically and psychologically abnormal, and these changes can wreak havoc on research outcomes. Behavioural management in general, and properly designed enrichment in particular, is the solution to this problem. However, designing enrichment that meets these goals is a difficult task, requiring real science, a central understanding of the animal's natural history and behaviour, and the ability to take the animal's point of view. Recent years have seen a number of powerful pertinent examples for laboratory mice (in addition to the many wonderful examples in the well-established zoo literature), and these were used to illustrate behavioural management and enrichment principles through the talk.

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ANZCCART New Zealand 2014 Animal Care Technicians' Individual Award

Setting up a guinea pig colony

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Step 1: Establishment of colony

Early in 2013 I was asked to develop a guinea pig breeding colony to support a new research programme at the University of Otago, Wellington. I had not previously worked with guinea pigs and they had never previously been bred at my facility. I was asked to identify and manage the requirements for a successful breeding colony, and work with the principle investigator (PI) to develop the research-specific components of animal care. This required extensive research and external consultation to ensure that the technical staff developed the skills needed to ensure optimal care of the animals and that the academic was appropriately supported with the development of a new experimental model.

I identified a number of problems during the initial establishment of the colony. Breeding stock acquired from another academic institution arrived in a very poor state. The animals were malnourished, and some were heavily pregnant (delivered within a week of arrival) and should not have been shipped. To help these animals recover we needed to immediately address their poor nutritional state. We corrected their dehydration with subcutaneous saline injections, and hand-fed them with a proprietary baby weaning food as a highly palatable diet for the first two weeks. Pregnant sows were group-housed and fed standard laboratory diet supplemented daily with hay and fresh vegetables. During pregnancy and lactation we minimised environmental stressors (excessive handling / noise pollution) and ensured their enclosures were enriched with a variety of guinea-pig-specific shelters and toys. With this approach, we were able to restore almost all animals to good physical health.

It also became apparent that no new genetic material had been added to the colony for at least six years. Our priority therefore was to introduce new breeding males (from Australia) to increase the genetic diversity and therefore the health of the colony. Once these unrelated breeding males were available we needed to establish a robust system to ensure that outbreeding of the colony stayed intact. I used the ROGY system described by the University of Otago, Dunedin, where there are four different groups, and each group is assigned its own colour (red, orange, green, yellow) (Figure 1). To ensure no inbreeding can occur, females move around the circle clockwise and males move across the circle when they become replacement breeders.

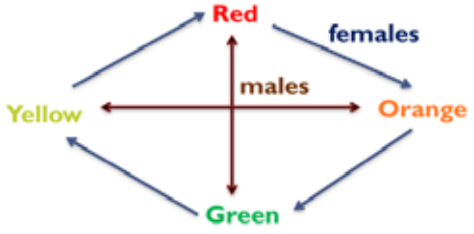

Fig. 1 ROGY mating system.

Step 2: Time mating

Unfortunately, information about prior breeding history for the pigs used to establish the colony proved to be inaccurate. An additional six months of monitored 'in-house' breeding was required to characterise normal healthy term gestation length. The research programme requires us to know the precise gestational age of all pregnant dams and pups. As guinea pigs are cyclic ovulators and usually breed in harems, there was little available information about the best technique for doing this. By close daily observation of our female guinea pigs, we developed the ability to reliably detect estrous via distinct vaginal openings, weight loss and erect nipples. We also took advantage of post-partum estrous which occurs 2-15 hours after the dams give birth. We configured our breeding pens so that at estrous females are placed with males for 24 hours; the lineage of all animals and precise gestational age is therefore known in all cases. After the dams have been with the males for 24 hours we check for the presence of semen which confirms copulation has occurred but not necessarily conception. After mating we palpate the abdomen to confirm pregnancy and estimate fetal numbers. Palpation of the abdomen can determine pregnancy from day 10. There are six discernible stages: Stage One (Days 10-15) – firm pea-sized swellings 5-6 mm; Stage Two (Days 15-25) – firm hazelnut-sized swellings 10-15 mm; Stage Three (Days 25-35) – elastic slightly oval bodies 15-30 mm; Stage Four (Days 35-45) – cylinder-shaped, heterogeneous bodies 3.5-5 cm; Stage Five (Days 45-55) – cylindrical fetus 5-7 cm; and Stage Six (Days 55-term) – fetus 7-10 cm.

Step 3: Induction of labour

Over the last year, my team and I have become confident in time mating guinea pigs and have a healthy colony which can supply time-mated guinea pigs according to the specific requirements of the academic staff. The research project requires preterm induction of labour (IOL) of the pregnant sows at very specific gestational ages to deliver viable premature pups. I have worked very closely with the PI to develop care pathways that support the development of the research programme, but ensure the well-being of the sows and their pups. We have sought regular input from the Animal Ethics Committee, including regular site visits, to ensure transparency of the high standards of care we offer our breeding stock, preterm and older animals.

At commencement of the IOL protocol (2 days before the day of birth) the sow is moved to a single pen where the IOL protocol is commenced. In the final stages, once uterine contraction has been stimulated, the sow is observed continuously until all pups and placentas are delivered.

Step 4: Care for the preterm pups

We based our care plan on the needs of newborn premature babies. We anticipated that the premature guinea pigs would require help with the following:

1. Temperature regulation – heat lamp, heat pad and plastic wrap (to reduce evaporative heat loss) were used to keep pups warm before transfer to the heated incubator.
2. Respiratory distress – initial lung inflation breaths and breathing support were provided using a 'Neopuff'™ infant T-piece resuscitator using blended air and oxygen. Additionally, the air within the incubator was humidified to minimise drying of the respiratory epithelium of the lung.
3. Nutritional needs of the pups – preterm pups cannot suckle independently. Artificial guinea pig colostrum replacement was drop-fed via syringe for the first 24 hours. Guinea pig milk was then provided until the pups were able to suckle (around day 4-5). Finally, pups were slowly introduced to weaning foods such as human baby food, vegetables, hay and pellets.
4. Maternal-pup bonding – it was important to make sure that the sow was able to bond with, and care for, her preterm pups. Pups were only removed from her for brief resuscitation as newborns, and all handing was done in the immediate vicinity of the sow.

High intensity guinea pig ICU care results in extremely low mortality rates. We have successfully reared 35 preterm pups with a survival of ~70%. The mortality that we have seen is due to failure of the pups to transition from fetus to newborn, inability to establish independent feeding, or maternal smothering of runts.

By developing the preterm guinea pig model in conjunction with the PI we have ensured that animal

welfare is prioritised by advocating the principals underpinning the 'Three Rs', whilst ensuring that the research needs of the academic staff have been met.

Upcoming projects for preterm versus term-born pups are as follows:

1. Behavioural and cognitive changes following preterm birth and effect of aging;
2. Disruption to the microcirculation following preterm birth;
3. Altered chronobiology and the late cardiovascular effects of prematurity;
4. Second hit hypothesis examining the combined or additive effects of preterm birth with the "Western Diet".

I have improved the welfare of the guinea pigs under my care by closely monitoring health and behaviour and responding to any abnormalities, modifying the animals' environment to match their specific needs and constantly refining practice to ensure optimal animal care. We have worked closely with the institutional veterinarian and developed close working relationships with local veterinary experts on guinea pig care. My team and I are confident in all aspects of time-mating guinea pigs and supporting the sows and their pups through preterm birth and postnatal care, and I have presented our work at ANZLAA.

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ANZCCART New Zealand 2014 Animal Care Technicians' Career Award

The challenges of change

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Straight from a veterinary nursing school in 2001, I began as Senior Animal Technician with the University of Otago, Christchurch School of Medicine, Christchurch Animal Research Area (CARA). This wasn't exactly what I had planned as a job after studying vet nursing, but I thought I could give it a try. During the 10 years I worked at the medical school I was directly involved in all aspects of the research, from surgical management to animal husbandry. There were opportunities to improve all standard levels in facility hygiene, animal care, husbandry, enrichment, improve pre- and post-surgical procedures, and surgical sterility.

Researchers and facility staff can become set in their ways, and change can be difficult to introduce. Good communication, leading by example and being directly involved with projects helped to earn trust. A collaborative programme of animal welfare refinement was finally implemented, which included significant changes in animal husbandry and monitoring which further promoted the welfare of the sheep used for biomedical research.

I implemented environmental enrichment procedures throughout all other species held at the University of Otago, including play rooms for rabbits, cage enrichment for rodents, and use of quality bedding, nesting material, feed and health monitoring. All this effort was visible through improved health and lower mortality throughout CARA's animal facility.

During my last two years at Otago University, the medical school suffered severe damage due to the Canterbury Earthquakes. I was extremely proud of the commitment my staff gave to ensure the animals were safe. Concentrating on the animals' well-being enabled me to cope with the hundreds of aftershocks we endured, always uncertain of the structural integrity of the old building that we and the animals were in; bits of concrete would regularly fall down around us, we were working in a yellow stickered building, and when it rocked we rocked too!

During this time I was offered a position to manage the Centre for Wildlife Management and Conservation at Lincoln University – a job that entailed even higher challenge: how to care for and handle captive feral animals so they thrive, and are healthy for experimental work. Getting wild animals to habituate to a captive environment with all the unknowns that come with them, such as no knowledge of age, pathogens and health, was quite different from my background of laboratory bred animals or farm animals.

I started by reading materials on how to improve the procurement, housing, care, feed, cleaning standards and enrichment for feral animals. I also talked to the manager of the Landcare Research facility which is in the same area as ours at Lincoln, so it was very handy to have Jane Arrow in the office next door; I need to thank her for sharing her knowledge with me. It is invaluable.

Firstly I needed some good staff. I asked if my animal technician Jan Gill from Otago University would like to work with me. She is an amazing animal technician with a vet nursing background, extremely

adaptable and willing, I cannot praise her enough! This was a new adventure for both of us.

I implemented a hygiene and sanitation regimen in both the inside and outside facilities. This instantly reduced the risk of disease outbreak. I also introduced new procedures regarding animal procurement, thorough animal checks and flea treatment on arrival, and protocols for sedative drugs which not only reduced stress to animals but also helped other students and post-doctoral researchers to handle animals. The result was less animal disturbance but still maintenance of adequate monitoring with clear routines and procedures.

Wild caught stoats were not surviving well housed in small cages in our inside facility. They suffered cage wounds to snouts, chins and teeth trying to escape through the wire. I implemented less noise by reducing foot traffic in the laboratory, interference of animals only when necessary, more privacy by covering cages and providing warm dacron bedding in nesting boxes. Mimicking a 'natural' diet for these fickle animals was essential and finding a yearlong supplier of fresh rabbit meat was also a challenge.

Newly built outdoor pens provided more space and survival success. Giving the stoats more cover in their outside cages by providing pens enriched with natural tussock grasses, hiding pipes, tunnels, branches for cover, nesting boxes raised off the damp ground and good cover from rain or sun decreased the mortality, giving a more natural type behaviour displayed for trials.

One of the best things an animal techician or laboratory manager can get is positive feedback. I very frequently heard from visiting scientists how much pleasure it is to see stoats happily hiding in our hanging tunnels. Most of the above procedures have now been mimicked with wild caught possums and wild rats.

A well run spotless facility, healthy animals and complete record keeping have been recognised by many, and makes me proud of my facility and staff wherever my career path takes me. Many of the above procedures have not only improved quality of life for animals, but have also saved lots of time and money for the research projects.

Being adaptable and accepting challenges has now led me to become involved in field trials run by the centre. We are testing resetting toxin delivery systems; these are species-specific, they are long-lasting - a 'set and forget' way of pest control. They can target low densities or high densities, and are a safer and more humane pest control method. I have been working in some of our most beautiful places and national parks. Our latest trial sees us at Totaranui in the Abel Tasman National Park, working on project "Janzsoon".

The last 13 years have been lots of hard work, but have also given me an enjoyable, rewarding and fulfilling career. I am most humbled to have won the ANZCCART New Zealand 2014 Animal Care Technicians' Career Award.

Research policy and the use of animals: (or) what's wrong with my mouse test?

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No full paper was provided.

This talk was based on a review written for the *ILAR Journal*: http://ilarjournal.oxfordjournals.org/content/55/3/438.full.pdf+html

Abstract

Three Rs work in the United States in particular has made great strides on Replacement (especially of toxicological techniques). However, the major ethical issue in animal experimentation, both in animal and human terms, is the lack of predictive validity of animal models. For instance, only 11% of compounds taken into the United States Food and Drug Administration (FDA) human trials succeed, and 80-90% of these failures are due to a lack of efficacy. Thus as each of these compounds 'worked' in an animal model, these models are producing an 80-90% false positive rate in early discovery. Improvements that weed out these false positives before animal toxicology and human trials would not only have the potential to drastically reduce animal use (Reduction), but would also alleviate the major financial cost in the pharmaceutical industry and thus have far reaching societal impacts. Much of the interest in 'biomarkers' from both US federal funding agencies and the international pharmaceutical industry represents the cornerstone of a new strategic approach to address these issues. In terms of animal models, this represents a paradigm shift in both philosophy and methodology; and one to which Three Rs approaches will be pivotal to success.

In this new era, individual variation, phenotypic plasticity, environmental and gene-by-environment effects are no longer viewed as noise that complicates animal models, but are embraced as the fundamental phenomena to be studied. Similarly, phenotypes with weak convergent face validity to human symptoms (e.g., 'anxiety-like' behaviours) cannot be justified; and highly determinant models (including many genetically modified (GM) mice) are of little use. Instead a new emphasis is placed on discriminant construct validity, by measuring in animals the very biomarkers shown in humans. The success of this Refinement approach requires Refined experimental designs, Refined biomarker-based measures, and Refined (i.e. enriched) housing and experimental conditions (e.g., supportive care).

This talk illustrated key reasons for the current false positive rates in animal models (interpretational issues in GM mice and determinism in models in general); methodological issues in GM mice; experimental design that ignores environmental interactions; 'phenotyping' approaches that emphasise efficiency over meaning; and the lack of enrichment and supportive care. Solutions to each of these issues, and their importance to biomarker-era models, were discussed, with special emphasis on the importance of enrichment. In this new 'biomarker era', "good welfare is good science" will be more true than it has ever been before.

From docking cradle, to baby cradle: paddock to bedside

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Abstract

A great many babies are born each day having been exposed to oxygen deprivation (hypoxia) during the process of birth which causes brain injury and life-long neurological disability. Until recently we had no treatment to help prevent or reduce neonatal brain injury. Now, for infants born full-term, we have one treatment called cerebral hypothermia (brain cooling). This treatment was developed using many different animal experimental models, but in particular fetal sheep by scientists in New Zealand. This treatment is now standard of care, worldwide, for the treatment of term newborn babies at risk of brain injury after oxygen deprivation at birth. Ongoing research seeks to refine this treatment, and to develop new treatments suitable for preterm babies for whom cooling remains potentially dangerous.

This review outlines why fetal sheep provide an important animal model for the study of perinatal clinical problems. It also examines why cooling, which was found to be neuroprotective more than half a century ago, took so long to be introduced into clinic, and how this relates to the fascinating history behind the development of the discipline of neonatology. This is a history which involves many animals, from chickens in a Paris Zoo to boxing kangaroos, and quite a few naked humans.

Introduction

Despite improvements in obstetric care, newborn infants continue to be born suffering from the adverse effects of oxygen deprivation (hypoxia) experienced before or during labour. Of the 130 million babies born each year, 4 million die before the first month of life, 50% die in the first day of life and 75% are dead within the first week (Lawn et al. 2005; Oestergaard et al. 2011). Hypoxia is a primary cause of their deaths (Lawn et al. 2005; Oestergaard et al. 2011). Long-term disability and illness is an issue for many infants who survive hypoxia. In the western world, where medical assistance is more readily available, around 2-6/1000 babies born at term will suffer brain injury related to hypoxia. If left untreated, approximately 60% of these infants will die or have long-term disabilities (Edwards et al. 2010). Those born preterm (<37 weeks) are at even greater risk of death and injury.

In New Zealand and Australia, around 8% of all of our births are preterm and this figure is rising, with the rate in the USA now 13% (Woythaler et al. 2011). Preterm birth is responsible for 70% of mortality and 75% of morbidity in the neonatal period (Oestergaard et al. 2011). These babies are more commonly exposed to hypoxia than term babies (Perlman 1997), and those that survive are at greater risk of neurodevelopmental impairment. Conservatively around 50% of all preterm babies will develop neurodevelopmental problems, with up to 20% of preterm infants at risk of severe injury and disability such as cerebral palsy (Woythaler et al. 2011).

These statistics mount up to one thing: lots of babies need our help to protect them from death, injury and

life-long disability. Thanks to experimental research undertaken using sheep (Gunn & Bennet 2009), and other animals, we now have the first brain protection treatment for term babies born oxygen deprived. This treatment is called cerebral hypothermia, or brain cooling (Gunn & Bennet 2010). This treatment is only available for mature babies, as cooling the body is a physiological challenge that in itself can cause illness and death if not controlled properly, and preterm babies are more susceptible to the cold. Thus, in its current use in term babies, it is not yet considered a viable treatment for them (Gunn & Bennet 2010).

Indeed, there were many clinical staff who felt that it was unwise to undertake this treatment in full term babies when the first safety trial was undertaken at National Women's hospital in Auckland, New Zealand, by Professors Tania Gunn and Alistair Jan Gunn in the late 1990s (Gunn et al. 1999a). Tania would frequently comment that staff would stop the cooling treatment early because they felt that the babies were at risk of death or that cooling was painful and distressing for them, an impression given because the babies shivered (*private correspondence*). The treatment of hypothermia was, they felt, unethical as it went against accepted standard clinical practice that stated that babies needed to be kept warm in order to reduce death and injury. Many were not convinced that just because something worked in sheep, of all creatures, this meant that sound clinical practice should be overridden! The nurses and other medical staff had every right to feel as they did because the standard therapy of keeping infants warm had a long history of evidence that it was effective at keeping babies safe. This is a history which also involves the use of animals and, with hindsight, some incredibly questionable human ethics.

The director of the Paris Zoo invented the first human infant incubator

It was the French who really established the clinical use of incubators for babies to keep them warm. In the late 19th century they had a major problem: they were running out of people. A falling birth rate (which was unique to France) and a high infant mortality rate, coupled with the loss of many adults in a few disastrous wars, left France with a dramatically declining population, which in turn brought with it significant economic pressures (Goldberg 1984; Corsini 1997; Baker 2000). Thus the challenge for French obstetricians was to develop ways to reduce the mortality rate which in the 1880s was 200-300/1000. This included trying to save the lives of infants born preterm or sickly. While term infant mortality was generally falling by the late 19th century, the preterm death rate remained high (>50%) (Baker 2000; Dunn 2002).

Leading this challenge was Etienne Stephane Tarnier, who was Chef de Clinique at the Maternity Hospital of Port-Royal in Paris (Baker 2000; Dunn 2002). In 1878, Tarnier visited the Paris Zoo to view the new *conveuses* (incubators) installed by the director of the Zoo, Monsieur Martin Odile, at the Jardin d'Acclimatation for the rearing of poultry (Editorial 1897). It struck Tarnier that such a device might also help his babies and he asked Odile to make him one to house human infants (Editorial 1897). Odile obliged, and the first was installed clinically in 1880 (Editoral 1897). Tarnier soon started to experiment with the design, replacing the paraffin heaters used in Odile's design with hot-water bottles, although his first attempts to get chickens to hatch using this method reportedly resulted in many hard-boiled eggs (Dunn 2002).

The incubators were a success, and in 1883 the clinicians published their findings on more than 500 babies, demonstrating that the use of incubators almost halved the rate of infant deaths (Auvard 1883). While clearly a good idea, getting other hospitals to use these incubators was not such an easy task. Innovations in the design of the incubator made them more sophisticated, but it also made them more expensive limiting their uptake by hospitals run by charities or limited government funding (Baker 2000). Inventor Frenchman Alexander Lion's incubator, which was considered the market leader, was one such example (Baker 2000). He financed his developments by setting up incubator charities which allowed sick babies to be cared for without charge, but he made money by allowing the public to come and see the babies for small financial consideration (Baker 2000). He also took his incubators to the commercial market, exhibiting them in the large industrial trade fairs of

the day which typically ran for six months to a year. The incubators were first exhibited in Berlin in 1896, where a rather interesting decision was made on how best to display the incubators: a decision which shaped the care of infants for the next 50 years.

Roll up, roll up, come see the tiny little babies!

In the Berlin Exposition in 1896, Lion and a colleague Martin Couney (who claimed to be a pupil of Tarnier's successor Budin) hit upon the idea that people would understand the purpose of the incubators better, and therefore their display would be far more popular, if they had living babies in them. Exactly who suggested this is not clear from historical documents. Lion clearly had a history of letting the public pay to look at sick babies in incubators, but Couney claimed the glory in later interviews (Silverman 1979; Baker 2000). They approached the director of the Berlin Charity Hospital, Rudolph Virchow, who 'donated' six babies for the show, on the grounds that they were going to die anyway, so nothing was to be lost (Silverman 1979; Baker 2000).

Today this would be equivalent to putting sick preterm babies on display in the Royal Easter show, not something we would consider ethical. However, this was a time when human ethics came from quite a different view point. Such trade exhibitions, and indeed zoos, frequently had people (slaves) on display in human zoos in ethnological exhibitions where you could go and see people in their so-called 'natural, primitive state'. It is a forgotten part of our human ethical history (http://www.popularresistance.org/deep-racism-the-forgotten-history-of-human-zoos/). At the Berlin Exhibition, the baby incubator display was next to the Congo village and Tyrolean yodellers and a parachute jump (Silverman 1979).

While used to seeing native people on display, it was quite something else to put a baby on show. The public could have been quite outraged. As it turned out, they loved the show, and songs were said to have been sung in beer halls about the *Die Kinderbrutenstalt* (the child hatchery) (Silverman 1979; Baker 2000). Thus began the long history of viewing sick and premature babies in incubators as an entertainment situated with other forms of amusements. Couney went on to lead what became known as the incubator side-shows throughout the first half of the 20th century (Silverman 1979; Baker 2000; Dunn 2002).

Elephants, Aunt Sallies, five-legged mules and dog-faced men

Couney was invited by the British showman Samuel Shenkein to bring his show to the 1987 Earls' Court Exposition in London. The show was an equal success, with more than 3600 people coming to see the babies on display in a single day (Editorial 1898). The prestigious medical journal *The Lancet* reported that the exhibition was well run and the medical advance that was the incubator was a welcome one in England given the preterm mortality rate of more than 40% (Editorial 1897). English physicians on the other hand were not impressed with the shows themselves and refused to provide babies for public display (Silverman 2002). Couney was forced to turn to his French colleagues for a supply of babies, who in turn obligingly shipped wicker baskets full of Parisian premature babies across the channel for his use. The reticence of English colleagues may have related to the many copy-cat exhibits around London which were not well run.

You could, for example, go see the babies on display in Islington at the agricultural hall and then pop by and see the Bostock-Wombwell Menagerie, which sported exotic wild cats such as leopards, and Consul the "Almost Human" Chimpanzee (who must have made his owners a lot of money for he was insured for £20,000, a princely sum even now) (http://www.georgewombwell.com/gw_blog/?p=308). When bored with that you could go to the Royal Aquarium where the babies vied for the public's attention with bicycle races, or go to Olympia where Barnum and Bailey gave you babies and the "Greatest show on Earth". The exhibition of babies as amusements next to what was perceived as clear health dangers posed by animals and the general mass known as the "public" angered many, as a Lancet editorial in 1898 entitled "The dangers of making a public show of incubators for babies" made very clear (Editorial 1898):

> *What connexion is there between this serious matter of saving human life and the bearded woman, the dog-faced man, the elephants, the performing horses and pigs, and the clowns and the penny peep-shows, and amidst the glare and noise of a vulgar fair?... Is it in keeping with the dignity of science that incubators and living babies should be exhibited amidst the aunt-sallies the merry-go-rounds, the five-legged mule, the wild animals, the clowns, penny peep-shows, and amidst the glare and noise of a vulgar fair?*
> (Editorial 1898)

The British medical profession and the public disagreed. The public loved the shows, returning time and time again to watch the babies grow. It was a money earner, and Couney clearly saw the potential. He immigrated to America in 1903, taking the incubators concept with him, and set up his own permanent show in Coney Island, where he exhibited infants every summer for the next 40 years. He exhibited his own prematurely born daughter, Hildegarde, who subsequently went on to become a nurse at the exhibition (Silverman 1979; Baker 2000). He also continued to display babies at the world trade fairs and expositions, both in the US and internationally (Silverman 1979; Baker 2000).

Boxing kangaroos, naked dancing ladies, pants on horses and explosive giraffes

In keeping with the entertainment theme rather than an educational one, the American exhibitions (which typically ran for a year) had the incubator shows in the pay-streak (or entertainment section) of the fairs. In 1901 at the Pan American Fair in Buffalo, New York, you could visit the babies and then go see a man in red tights fight a boxing kangaroo. In the Alaska-Yukon fair of 1908, you could go see the babies and then go next door to see the wild west show featuring Princess Trixie the talking horse. By 1933, in Chicago, there were fewer animals and more "oddity shows" such as Rippley's Believe it or Not Odditorium, where a man pulled a girl in a cart using his eye-lashes, and a woman showed she could swallow her own nose (Ganz 2012). There were also naked dancing ladies.

The incubator show was housed next to the Sally Rand show, where you could go see Sally and her exotic semi-nude ladies dancing with large feather fans. Huge crowds flocked to see Sally and her girls, and in one week they reportedly earned $75,000 (Ganz 2012). Others were morally outraged and Sally and her girls were arrested at least four times, on the basis that their lewd and lascivious behaviour would corrupt the morals of the delicate infants and indeed the wholesome morals of the families who came to be entertained at the fair as a whole (Ganz 2012). Not all agreed that anyone's morals were at risk, and on one celebrated occasion the judge, Superior Judge Joseph B David, threw out the case stating:

> *There is no harm and certainly no injury to public morals when the human body is exposed, some people probably would want to put pants on a horse … If a woman wiggles with a fan, it is not the business of this court. When I go to the fair, I go to see the exhibits and perhaps to enjoy a little beer. ... Case dismissed for want of equity.* (Ganz 2012)

The last time the babies were on display was at the 1939-1940 New York World Fair. Here at least one could be educated by the American Natural History Museum which bordered one side of the incubator exhibition, but the other side had a peepshow called "Enchanted Forest". Further down the road one could be treated to surrealist naked ladies in the Salvador Dali exhibition, "Dream of Venus", along with paintings of giraffes on fire (Schaffner 2002). It could have been worse; Dali first planned on having a more spectacular show! In 1939, while preparing his exhibition, he wrote to his friend the Spanish cinematographer, Luis Bunuel saying "Here I'm designing a Surrealist pavilion for the World's Fair with genuine explosive giraffes" (http://www.brooklynrail.org/2003/11/artseen/salvador-dals-dream-of-venus).

Money made, but babies were saved and neonatology was born

By this time the public had lost its appetite for such shows, and there was medical pressure to have babies treated only in hospital. We look back at these incubator side-shows with a touch of moral horror. How could people have condoned such an act as to put a baby, never mind a sick preterm baby, on show, day after day, for the sole amusement of the public?

In later years Couney claimed that he only did this for the love of the babies (Silverman 1979; Baker 2000). There is no doubt he significantly contributed to the development of the disciplines of neonatology and paediatrics, fields of medicine which did not exist then. It was the staff who ran the incubator shows who developed the technology and the clinical protocols which kept such fragile babies alive, working out their nutrition and oxygen needs in particular (Baker 2000). In truth, these side-shows were functioning for the most part as high-end neonatal units, because the

money to be made was in repeat business. People came back to see their favourite babies growing up. To make money, you needed babies to survive. The protocols they developed were then taught to the medics and nurses, such as the renowned Evelyn Lundeen from the Michael Reese Hospital in Chicago, who eventually oversaw their introduction into hospitals (Lundeen 1954).

By the 1950s, randomised control trials were undertaken by Bill Silverman and colleagues to prove, clinically, what the Paris Zoo director Odile already knew in the 19th century: warmth improves the survival of newborn babies (Silverman et al. 1958). Thus was born the straightforward clinical mantra "warm babies good, cold babies bad", which stood in the way of getting brain cooling into clinical trial.

Being cold: kill or cure

Being too cold can make you ill and can kill you. This is something we all understand, but there is a long history of evidence to show that the judicious application of cold can also be therapeutic, even under the most bizarre of circumstances such as drowning in cold lakes and even hangings (Karnatovskaia et al. 2014). Take the curious tale of Annie Greene, for example. Annie was found guilty of killing her own newborn infant, an infant likely prematurely stillborn and one she did not know she carried, and was sentenced to death (Breathnach & Moynihan 2009). She was hanged on a cold winter's morning, but when cut down 30 minutes later was observed to have survived (by the man who had paid a fee for the opportunity to dissect her). Doctors attended her and revived her with warmth and all manner of cordials and poultices. Within a month she went home a free woman, pardoned of her crimes. She subsequently married and had three further children (Breathnach & Moynihan 2009).

Physiologists, such as James Curry and John Hunter, began experimenting on the effects of cold in the 17th and 18th centuries (Guly 2011; Karnatovskaia et al. 2014), and in the 19th century Dominique Jean Larrey, surgeon-in-chief to Napoleon Bonaparte, described in detail how the cold affected injured soldiers, and that while invariably deleterious, sometimes it was beneficial, commenting "Soldiers lying closer to the campfire died sooner than those in more remote, colder areas"(Larrey 1814). However, it was not really until the 20th century that the benefits of hypothermia were fully studied. Dr Temple Fey is credited with its reintroduction to medicine for treatment of cancer and a variety of brain injuries (Karnatovskaia et al. 2014). In the 1960s, this work was taken further by many including Peter Safar and colleagues, who demonstrated in animals and humans that mild therapeutic hypothermia might be neuroprotective in cases of stroke or coma after cardiac arrest (Thoresen 2000; Kochanek 2009). Today, there many ongoing trials for stroke and cardiac arrest patients (Karnatovskaia et al. 2014).

For neonates, as for adults, the development as a neuroprotection treatment for newborn babies and adults also came from a parallel history of both human and animal experiments. Dunking babies in cold water as a method of resuscitation of still-born infants had been used for centuries, and indeed was still in use until the 1930s (O'Donnell et al. 2006). In the 1940s Miller and colleagues demonstrated that newborn animals who were cold survived oxygen deprivation for longer (Millar 1949) and, from this work and other studies, he and his colleague Westin went on to trial brain cooling on newborn infants (Westin et al. 1959). The authors reported that the cooled babies appeared to have better neurological outcomes and a follow-up many years later showed the children did not have major handicaps like cerebral palsy (Westin 1971).

Both Westin and Miller continued their work in animals, and carried out several other small-scale human trials well into the 1970s (Wyatt & Thorsesn 1997). However, despite their work, and evidence of efficacy in adults, hypothermia as a treatment was not formally assessed as a therapy, and the concept was abandoned until the 1990s. Arguably, it only took one scientific paper to sink the concept of brain cooling in infants, and that was the paper by Silverman and colleagues showing that warming babies saved their lives (Silverman et al. 1958); that and the bizarre history of side-show incubators of course. The idea of "cold babies bad" became a very entrenched clinical paradigm (Wyatt & Thorsesn 1997).

It took some Kiwi scientists to revive the idea in the 1990s (Gunn & Bennet 2010), and Silverman himself (the man who clinically determined warming babies was the way to go) to weigh in on a decade of debate about how to balance the good and bad when it came to cooling newborns (Silverman 1998, 2002).

Does it matter if babies get a little cold?

In the 1980s and 90s, Auckland neonatologist and scientist Tania Gunn had an interest in studying which physiological mechanisms switched on temperature regulation at birth. At the same time her son, paediatrician and scientist Alistair Jan Gunn, was undertaking PhD studies on developing brain protection treatments for newborn infants. His research had shown him that many drug treatments which provide some degree of brain protection after hypoxia also cooled the brain. However, brain protection was not improved if cooling induced by these drugs was prevented. Rather, protection was reduced or lost. Cooling rather than the drugs themselves, it seemed, might be the protective factor. Alistair and Tania revisited the history of cooling and realised that there were in fact a surprising number of experimental and clinical data strongly suggesting that hypothermia was beneficial. They resolved to revisit the question experimentally despite the fact that they had been taught as clinicians that cold was bad for babies. They reasoned, however, that if their experiments proved hypothermia was neuroprotective, then the bad effects of cold could be managed in a modern day clinical environment. The brain could be cooled, but the rest of the body kept reasonably warm (*personal correspondence with the Gunns*).

They chose for their studies the animal model of the chronically instrumented fetal sheep. In this model ewes are anaesthetised and, under sterile surgical conditions, a laparotomy is performed to expose the uterus. The uterus is opened, and parts of the fetus extracted (the head and chest for example) for the placement of catheters, electrodes and other monitoring probes for subsequent monitoring of fetal cardiac function, and brain and body behaviour, and for blood sampling. A soft silicone occluder can be placed around the carotid arteries or umbilical cord for later use to restrict blood flow to the fetus (to induce hypoxia). When surgical procedures are completed, the fetus is returned to the uterus, and the laparotomy wound of the uterus and the ewes repaired (think of it as a reverse caesarean). All of the catheters and other tubes are exteriorised though a small hole in the ewe's flank so they are out of her way. The ewe is woken up, and returned to a metabolic crate where her fetus can be studied growing and developing without the confounding effects of anaesthesia.

Using a fetal subject when the clinical target is a newborn may seem strange. However, this model offers many advantages in such studies. Principally it allows scientists to study their subject using an integrated systems physiology approach. They can measure many physiological and blood chemistry variables at once continuously and thus integrate knowledge about many physiological and pathological processes – just as one does clinically. This gives us more power to develop effective therapies, and to understand why treatments may be effective, and to permit scientists to quickly dismiss those which are not safe, or which are ineffective. It also allows scientists to study the animal without the confounding effects of anaesthesia and other clinical treatments which would be necessary if we delivered the animals and studied them after birth. These confounders act to mask our understanding about normal and abnormal physiology, and the effect of treatments independent of clinical management treatments. For ethical reasons, there is also a very limited period of time to study sick newborn animals and thus considerable information is lost about how brain injury develops over time.

This latter point is vital, as the reason why we can give a newborn baby (and us as adults) a neuroprotection treatment is because injury evolves over time (Gunn & Bennet 2008; Bennet et al. 2009, 2010). Most brain cells don't die during the period of oxygen deprivation, they die in the hours to days to weeks after the insult in distinct phases (Gunn & Bennet 2008; Bennet et al. 2009, 2010). Thus this gives us time to treat. Considerable work was undertaken during the development of the brain cooling treatment, and is ongoing now, on understanding the adaptations to hypoxia, the pathological processes mediating the phases of injury after hypoxia, and therefore how cooling (and other treatments) could prevent the factors causing injury. Many studies were also required to determine when to start, how long to treat, and the degree of cold to use and how fast to rewarm. In a seminal series of studies, the powerful efficacy of cooling was established in fetal sheep along with the parameters of the treatment protocol which could be translated to the clinic (Gunn et al. 1997, 1998b, 1999b,c).

Key factors were determined about its use: 1) the treatment was most effective when started early after

the end of an insult, the earlier the better; 2) efficacy of treatment was lost if treatment started after six hours; 3) cooling needed to be continued for at least two days, but three was better (new studies from our laboratory suggest that longer treatment is not more beneficial, so three days is about right); 4) there is a dose response curve for cooling – you can be too cold (which can cause damage, and was a confounder for many earlier experiments) and you can be not cold enough (too warm, and treatment efficacy was lost); 5) cooling does have potentially dangerous effects on the heart, oxygenation and some blood chemistry which must be monitored; and 6) slow rewarming is essential to prevent adverse events such as rebound seizures.

So it took many experiments to establish how to use cooling. Armed with this knowledge they proposed a clinical safety trial, designed to cool the brain but use an overhead heater to minimise the negative effects of cooling. The protocol used on humans is pretty much the same one determined in sheep. However, despite the evidence which mounted from the sheep experiments, showing that early cooling could save a significant number of brain cells from dying, debate raged at scientific and clinical conferences on the topic of whether to trial this in humans. Silverman penned a commentary in the scientific journal *Pediatrics* decrying the rigidity of thinking on the subject, stating:

> *The hoary intervention for total-body hypothermia was first mooted 300 years ago. A proper trial (with concurrent controls) of the latter intervention should have been carried out at least 35 years ago: the mills of the gods do, it seems, grind very slowly indeed. ... It's outrageous to keep guessing for 300 years about whether or not "infants are 'meant' to get a little cold after birth". The question is eminently testable!* (Silverman 1998)

The question was testable, and after assessing properly in appropriately designed animals, the Gunns carried out a safety trial. Tania's lovely, but persistent nature won over the medics and nurses and the trial proved that cooling could be used safely, so long as strict protocols were followed (Gunn et al. 1998a).

Around the world researchers began to study the question more intensively, and this work culminated in an international multi-centre, randomised control trial led by Alistair Jan Gunn, the results of which were published in 2006 (Gluckman et al. 2006). The study concluded that cooling significantly reduced neurodevelopmental disability for many babies as assessed at 18 months of age. Since then, six major trials have confirmed the finding (Edwards et al. 2010; Laptook 2014), and it has recently been reported that protection is still seen in seven-year-old children (Azzopardi et al. 2014). This treatment is now a standard therapy for term neonates born hypoxic at birth.

The future?

This is the only neuroprotection treatment available for newborn infants and it is only available for term newborns. Our laboratory (the Fetal Physiology and Neuroscience group led by myself and Alistair Jan Gunn) has shown that the preterm brain, which is at much greater risk of injury, does have a similar evolution of injury after hypoxia, and that cooling can prevent injury (Bennet et al. 2006, 2010; Gunn & Bennet 2008). However, as the French observed in the 1880s preterm babies are more vulnerable to the cold, and clinically, on the basis of legitimate safety concerns, there is resistance to instituting brain cooling as a therapy for preterm babies (Gunn & Bennet 2010). Research is now ongoing to find a treatment that could be given with milder cooling, or which is therapeutic in its own right and which would be safe for babies of all ages. Melatonin and erythropoietin are amongst the leading candidates currently being tested (Robertson et al. 2012).

The preterm brain must also be studied from the perspective of neurorepair rather than neuroprotection as clinical and experimental data tell us that by the time preterm babies are born they have long passed the window of opportunity for neuroprotection (Bennet et al. 2013). Here such therapies as stem cells may help repair and regrow the brain (Bennet et al. 2012). Equally, such approaches may benefit term newborns who do not benefit from cooling because they too may have experienced hypoxia well before birth; thus by the time they are born their brain has evolved too far along the injury pathway (Bennet et al. 2010).

New research is also required to begin layering our knowledge of the physiological and pathophysiological responses to insults like hypoxia, with information about how other insults, such as infection occurring at the same time, may change

responses to hypoxia. Preterm infants, for example, are often born with infection and hypoxia. Similarly, we need to understand how other standard clinical treatments, such as clinical drugs like steroids, sedatives and anti-seizure drugs, and recreational drugs taken by mum and dad, also affect the responses to insults and to treatments. Such interactions have the capacity to change the responses to injury and to treatments.

Such challenges are immense, but the strange journey of temperature and newborns related to you in this conference paper has taught us many things. Key amongst these lessons is that effective treatments can be developed if we understand basic physiological and pathological processes, undertake good animal and human experiments, and keep our minds open to questioning and challenging fixed perceptions and ideas. Sadly Tania died before the international clinical trial of brain cooling was undertaken, but many children owe their lives and their intact minds to her scientific and clinical acumen, and her flexibility of thinking, and it is to Tania that this review is dedicated.

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Investigating lead poisoning in kea

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No full paper was provided.

Abstract

The New Zealand alpine parrot, the kea (*Nestor notabilis*), is notorious for its bold and investigative behaviour. This can result in exposure to a number of anthropogenic (human-caused) hazards including lead, a toxic heavy metal. A survey was set up to expand on previous work which showed high blood lead levels in kea around an alpine village. Between 2006 and 2009, kea were captured in various parts of their range and samples of their blood were taken for blood lead analysis. This study examined whether kea from areas with permanent human settlements experience a significantly higher level of exposure to lead than those in remote areas. The results of this study were presented and the implications for the conservation of this species discussed.

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Ruminating on ruminants: if I were a sheep...

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Abstract

Sheep are very common farm animals in New Zealand and also make up a reasonable proportion of animals used in research, testing and teaching. New Zealand sheep farming's reputation for very good animal welfare is well known, but the public perception of animals used for research is not always as positive.

This presentation aimed to compare the welfare experiences of sheep in the farming sector with those of animals used in research, testing and teaching. If I were a sheep, would I rather be on a farm or in a research trial?

Background

The practice of science discourages anthropomorphic approaches to problems, preferring conclusions founded on the interpretation of data sets gathered from carefully designed experimental studies. This is such an ingrained attitude that some definitions of science specify the experimental method as a prerequisite for something to be considered scientific (Thompson 1995). Despite this, it is sometimes necessary to form opinions about issues that cannot currently be resolved using the scientific method either because such issues are not well enough understood to be able to design appropriate experimental methodology or because the issues are fundamentally outside the realm of experimental methodology. Sentience and the subjective experiences of animals are currently such issues. The last few decades have seen the beginnings of the application of experimental methodology in these areas, but we are not yet at a stage where we can fully understand the subjective experience of non-human animals.

Even in areas where experimental data are not available, society as a whole looks to scientists to develop informed opinion and to form a collective view as to the likely situation. In forming this opinion, the scientific community needs to consider the available data as well as extrapolating by use of techniques such as anthropomorphism that clearly lie outside the usual scientific toolbox. It is not currently possible to ask "If I were a sheep, would I rather be on a farm or in a research study?" without some degree of anthropomorphism.

This paper informally explores how the public form their attitudes about animal use and the ways in which we can develop informed opinion as to the acceptability of using animals in production and research systems.

Attitudes to animal use

The general public encompasses a spread of opinion as to the acceptability of utilising animals for human benefit. At the extremes are contrasting views on the one hand of animals as chattels, subject to no inherent value other than their monetary worth (Fellenz 2007) and deserving of no protections; and on the other hand, of animals as ends in themselves, worthy of such consideration that any exploitation for human benefit is completely unacceptable (Regan 1989). The vast majority of opinion falls between these two extremes and can be illustrated by considering the position of organisations with an interest in the

use of animals in farming and research along a scale from pro to anti. Such a consideration is illustrated in Figure 1. In general, organisations that are pro the use of animals in farming are also pro their use in research, but organisations that are against research use are not necessarily against farming. Organisations that are against the use of animals in farming, but not research usually have a specific agenda such as the promulgation of a vegan lifestyle. This imbalance suggests that, in general, public opinion tends to view the use of animals in research as more of a concern than the use of animals in farming.

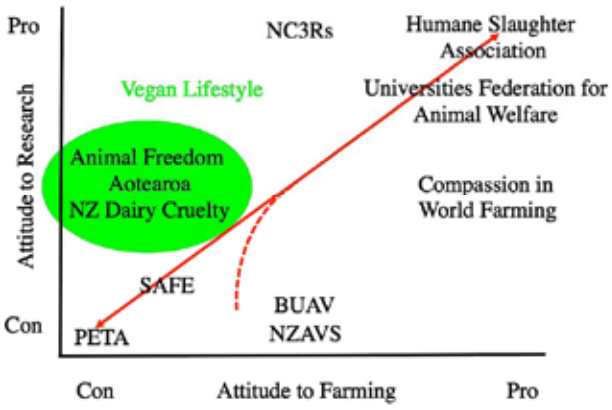

Fig 1 Attitudes to farming and research of organisations reflecting public opinion on these issues. NC3Rs: National Centre for the 3Rs. BUAV: British Union for the Abolition of Vivisection. NZAVS: New Zealand Anti-Vivisection Society. SAFE: Save Animals From Exploitation. PETA: People for the Ethical Treatment of Animals. Attitudes derived from information on organisation websites.

Justifications for farming and research

The justifications for use of sheep in farming are based around the utility of products such as meat and wool. Similarly, the justifications for the use of sheep as research animals are based around the utility of the data that are derived from such experiments. Humans eat meat derived from sheep and wear clothing made from wool; they gain benefit from research in terms of improvements in farming systems and public health. One would imagine that the sheep are not heavily invested in any of these benefits and would be more concerned about the impacts to their welfare of being involved in farming or research systems. The remainder of this paper considers only the impact on the sheep of research and farming and then attempts to compare these.

The sheep as a research animal

When sheep are kept as research animals, individual animals are often of much greater economic value than farmed animals. This increase in value may cause them to live in a more comfortable environment and these two factors together may contribute to a greater life expectancy compared to sheep on a farm. In contrast to these improved living conditions, sheep such as these may have been exposed to an event or series of events that had increased impact on their welfare. A good example of this is a ewe with a fistulated rumen. Such animals can have very extended lifespans compared to animals on farms, living to as much as 12 or 14 years of age. They are not required to breed and are provided with good quality food and shelter during poor weather conditions. This must be balanced against the surgical procedure of rumenal fistulation and the inevitable period of weight loss as the animal's rumenal micro-organisms adapt to the altered conditions of the fistulated rumen. Although fistulation will be carried out under anaesthesia with the provision of post-operative pain relief, there is some welfare cost to the animal in being subjected to such a procedure.

Compared to animals of many other species, sheep tend to be involved in research studies with relatively low welfare impact. In order to facilitate comparison between species, figures were taken for animal use in 2012 (Anon. 2012) – the most recent available at the time of writing – and a comparative ratio of animal impact groups (CRAIG) derived for each species or group using the following formula:

A : [(B*2) + (C*3) + (D*4) + (E*5)]

where:

A is the number of animals in the "No Impact" group;

B is the number of animals in the "Little Impact" group;

C is the number of animals in the "Moderate Impact" group;

D is the number of animals in the "High Impact" group; and

E is the number of animals in the "Very High Impact" group.

The results of this analysis are illustrated in Figure 2 and demonstrate that when sheep are involved in research, they are more likely than any other group of animals (except dogs) to be involved in research with a low impact category.

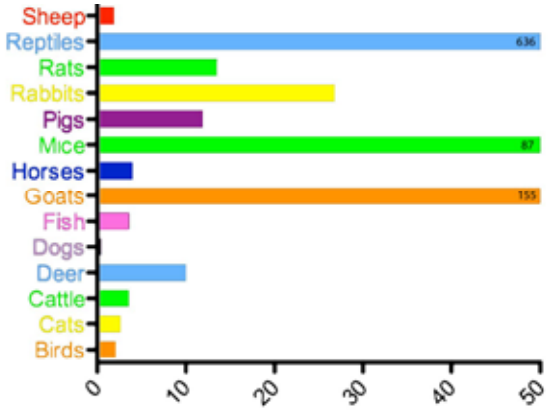

Fig 2 Comparative ratio of animal impact groups (CRAIG) derived from National Animal Ethics Advisory Committee annual report 2012 (Anon. 2012). See text for explanation of CRAIG.

The sheep as a farm animal

Sheep on farms are of moderate individual economic value, derived from their ability to produce wool and to reproduce regularly. Sheep often live in exposed environments with little shelter and relatively poor quality nutrition. The environment combined with the need to remain productive often limits the lifespan of ewes on farms to as little as four years. Many farmed sheep undergo surgical procedures such as castration and tail docking, and the need to limit costs means that these procedures are often carried out with little or no provision of pain relief. Whilst these welfare issues are authorised by Codes of Welfare, they can represent significant impacts on the welfare of the animals and need to be taken account of in any comparison with research animals.

The more realistic question

Even though sheep are often subjected to farming in relatively harsh environments and more usually subjected to research of a relatively low welfare impact, it must be remembered that the majority of sheep that enter research projects are sourced from a farming environment. They will usually return to the farming environment following the research and may remain on the farm for the duration of the research study. Even though the research to which these animals are subjected is of minor welfare impact, the lifetime experience of these animals will also include the 'normal' welfare costs associated with farming. In these cases a more realistic question to ask of these animals would be "If I were a sheep, would I notice any difference if I was on a farm or in a research study?"

Conclusions

Public perceptions of farming and animal research can be very different, with farming seeming to have a greater degree of acceptance and a better image than animal research. In reality, most sheep subjected to research would appear to incur little additional welfare impact beyond that already experienced due to normal farming practice. For small groups of animals, association with research programmes will incur animal welfare impacts at the start of the research, often due to surgical manipulations, but there will often be long-term benefits to these animals in terms of improved environments and extended lifespans.

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Getting the most out of commercial farm trials from a design, ethical and scientific perspective

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Commercially funded trials involving production animals may involve a wide variety of clients ranging from pharmaceutical companies to animal nutrition companies. Manipulations can range from those common to normal farm practice to quite invasive procedures and while some may be performed 'on farm' others may require management of animals in indoor pens or cages within a research facility. The majority of the commercially sponsored trials we have worked on require that animals used are returned to the owner/food chain, with the minority requiring special containment, withholding or euthanasia and disposal conditions.

Having been involved in commercial farm trials for a few years now, a key lesson we have learnt is to scope out a customer's requirements with the aid of a check list of considerations, and to assume very little. It is imperative to take the time to give due and careful consideration to any proposal from a commercial client: there can be a disconnect between what a client expects and the realities of robust and well-defined research.

Care must be taken to understand what 'horizon' the research occupies, is it discovery, translational or implementation? Has a 'problem' been created for a 'ready-made' solution? Commercially driven research may have restricted objectives linked to market and drive sales of test products; for research providers more accustomed to working on grant-based research this may create some issues with defining a clear proposal without customary mechanistic objectives. This is especially important for research involving the animal production industry and may help indicate as to where the research should be conducted, under what conditions, and involving which manipulations. In our experience, some research provision is managed via a company's marketing division, with the key driver for the research being to drive sales and market dominance of the company's product. Ensuring that funding for a trial is sufficient with contingencies built in can be tricky to negotiate: before committing to a commercial trial a budget must be agreed to with clear expectations laid out in a contract.

All work must be compliant with the available welfare codes, developed alongside the Animal Welfare Act (1999). Commercial research must comply with the research provider's institutional animal research regulations and may require specific ethical approval if the practices used deviate from normal farming practices. This can be a grey area and it is prudent to consult the institutional Animal Welfare Officer and/or Animal Ethics Committee (AEC) about their perception of whether ethical approval is required before progressing too far with trial planning and protocol development. Routine interventions performed by farm animal veterinarians for the monitoring (e.g., blood sampling or biopsy) and treatment (e.g., ambulatory surgery) of sick and/or injured animals may be more invasive than many basic experimental procedures.

Ensuring the balance between experimental work and routine veterinary procedures is an exercise that requires diplomacy, transparency and open communication. We have found that meeting the

property owner's veterinarian prior to starting a trial is invaluable; this ensures that they are fully aware of the trial's focus and they are in an excellent position to flag any potential issues. Owners of farm animals and clients are unlikely to have a clear idea of what requires ethical approval and what does not. Further on from this, in our experience, commercial clients generally have little understanding of what goes into writing an animal ethics application; there may also be an attitude that they can circumvent the ethics process. The researcher has the responsibility to make sure they and their client have considered all ethical and welfare considerations. Regardless of adherence to 'normal farm practice' we have now reached the conclusion that animal ethics approval must be sought to ensure the trial is run to the best standards possible and so the client does not take excessive licence from a previous experience. The added bonus of the exercise is that design and statistical considerations will undergo additional independent review as part of the ethics approval process. This also protects the reputation of the parent institution the researcher may belong to – these are broader ethical issues beyond animal welfare.

Any contractual agreement must decisively address whether the client requires a peer-reviewed publication written at the conclusion of the trial as this may have a huge impact on the scope and cost of a trial. In a country like New Zealand there is a strong consideration given by journal reviewers to the range of ecotypes and systems nationally and a desire expressed that trials are large and representative – either from a scientific or industry perspective. Satisfying statistical power in a limited or smaller setting may not be enough. This is particularly important beyond the discovery research horizon and extends into translational implementation research horizons. From experience, it is best that once the decision has been made regarding limiting scope of dissemination (e.g., to reports and newspaper articles), revising the decision to publish in peer reviewed journals after the fact should be avoided where possible. There is a need to be wary of drive by a client to turn a small trial into a peer-reviewed paper: this is easier said than done, and clients may need to face the fact that we are at the mercy of a journal's editors and review panel. Simply paying for a paper to be written does not guarantee acceptance for publication.

Running trials remotely (e.g., farm is in a different part of country) creates a plethora of additional issues surrounding animal welfare, e.g., regular animal monitoring, interventions, and euthanasia situations that need to be flagged and discussed before a trial begins. With the case of lambing survival trials, 'easy care' lambing (i.e. minimal intervention) is currently the norm on large farms; this is a clear departure from how we are used to running controlled lambing experiments so clear communication is required to ensure that any data collected will stand up to any welfare scrutiny.

For trials where the researcher controls animal sourcing, existing standard procedures used for other research (e.g., Public Good Funded) are a good base for operation with attention paid to animal health, age, etc. For large animal-based research, breed and genetic background may be of paramount importance for the research undertaken and may have ethical and welfare considerations (e.g., sheep breed and facial eczema research) as well as trial outcomes. Production animals may also be quite outbred, especially on a client farm. The researcher must gain a clear view of how this variation may affect the power of the research undertaken and take account of this in experimental design. Statistical constraints must be communicated very clearly with clients to also make sure the importance of randomisation and contemporaneous control groups is well understood.

Execution of a trial is usually straight forward when working with staff/colleagues you have past experience with. However, where the work is performed on a client's property no assumptions should be made about, for example, animal health considerations or any of the normal expectations for nutritional management and practices to limit the effects of behavioural stress.

We have provided bullet point outlines of the two trials portrayed in our presentation to demonstrate the variety of requirements and considerations that may be encountered. The first was a highly controlled pilot toxicology trial with clear departure from normal farm practice. The second trial was on a farm with no departure from normal farm practice and very close to the translational stage.

Case Study 1: Testing of mycotoxic binders after Sporidesmin (SP) intoxification

(See Di Menna et al. (2009) for a description of the problem this research was addressing.)

- AEC approval sought and achieved – SP dosing in this case is seen as a departure from the licenced use for ram selection. In addition, SP dosing is an invasive manipulation.
- The AEC requested that dosing veterinarian be listed as a co-investigator for the trial.
- SP dosing performed by a New Zealand registered veterinarian, as requested by the SP supplier.
- Targeted doses and animal number based on prior knowledge of flock genetics and SP tolerance: power tested.
- Trial run and managed by University of Auckland staff and Auckland UniServices consultants (senior scientist and large animal vet nurse/research technician), within a University of Auckland animal facility.
- Data collected and managed by consultants performing data analysis: data were tightly controlled and very robust ('1st party data').
- Regular blood sampling to monitor liver biochemistry done by consultants.
- Novel test agents used: import licence also required through Ministry for Primary Industries; AEC approval required before import licence gained.
- Pilot type study: short time frame due to budgetary and time constraints.
- Outcome: further development of agents required, and more detailed and longer study recommended.

Case Study 2: Ewe and lamb survival

(See Everett-Hincks & Dodds (2008) for a description of this issue.)

- A single Southland model farm: approximately 300 triplet-bearing ewes/year that were the focus of the trial. Mix of singles and twins as well. Excellent grazing/pasture management.
- Use the 'easy care' model: low intervention style of farming. Farm owners had strong belief that intervention causes mis-mothering.
- No formal examination of 'ewe behaviour': farm owner comments purely anecdotal and not based on any formal observations.
- Pregnancy scanning to establish lambing numbers. Live lambs at weaning calculated against scanning rates. No tagging of lambs until weaning.
- Purely observational trial testing a variety of ewe supplementation just prior to and after lambing. Short time frame trial due to timing and budgetary constraints.
- No blood sampling (ewe or lamb) or euthanasia for tissue collection/analysis.
- Product marketed overseas, no departure from farming practice so AEC approval not sought in this instance.
- Contractual inclusion made that all 'on farm' practices were consistent with relevant New Zealand welfare codes developed under the Animal Welfare Act 1999.
- Trial managed remotely by Auckland UniServices consultants.
- Local veterinarian subcontracted for necropsy of dead lambs. In case of multiple deaths within a litter, a lamb selected at random for necropsy and cause of death estimated. While data were detailed it was very open to debate as to true cause of death: some categories overlap. Very limited budget available for post mortem examination, time constraints.
- No histological analysis was performed.
- No ewe post mortems performed: farm owners made estimates as to cause of death.
- Weather records, pasture analysis available for report write up.
- Despite being remotely run, research inputs required: randomisation of treatment groups, data management and consolidation, statistical analysis and final report.
- 'Mild' lambing season – possibly not representative of the range of conditions as it would ideally be.
- Consultants given '3rd party data' to triage and interpret: very time consuming and required extensive verification.
- Outcomes: promising within constraints and data noise.
- Animal welfare costs and perceptions must be weighed against the industry dogma of 'easy care' as a philosophy. Considerations between high stocking rates / multiple-bearing ewes / potentially higher losses / ethos of 'easy care' lambing. Is it really worth it? Is it false economy and perhaps a poorer welfare option?

Conclusions

The case studies illustrate some of our experiences with commercial research trials. Of note is the comparison between a trial run at our own facility (example 1) and a remotely run trial (example 2). Ensuring any commercial research is run and managed in a rigorous manner is of primary importance in ethics – in both an animal-specific and broader sense. The ability to control and manage data is paramount; however, it is also equally important to run these sorts of trials on-farm where possible, especially if the trial is an attempt to enhance a current farming system. Trial 1 provided a great deal of pilot information for the client to consider whether product development should continue and did not require the use of large numbers of sheep or highly compromising doses of toxin. Trial 2, while promising, may not address the issues of inter-farm variability or climatic extremes; but it has informed the client that they should get more comprehensive data from additional farms. We only had one visit to the farm and realistically much of the data noise could have been avoided with more regular scrutiny of collection. Reports from each trial to the client clearly identified the limitations apparent above and also opportunities for further research.

Finally, commercial research is a good opportunity to engage with the wider farming and production animal business community – after all, animal welfare doesn't start and stop with AEC approval.

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The tortuous journey from bench to bedside: exploring the contribution of animal research

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Abstract

The use of animals in research rests on a utilitarian argument that the cost in terms of animal welfare is justified by the potential benefits to humans and other animals. This argument is often supported by examples such as vaccines, transplantation surgery and some cancer therapies where the link between animal research and clinical application seems clear. But a closer look at the discovery process reveals that in most cases, the journey 'from bench to bedside' is far more complex. In this presentation, the story behind one of the most revolutionary technologies in biomedicine – monoclonal antibodies – was used to illustrate how progress comes not just from animal research, but from the coming together of complementary methodologies some of which are often advocated as alternatives to animals. The implications for Animal Ethics Committees when making a judgment on whether the potential benefits of a project are likely to justify the costs were discussed.

On 10 December 1901, Oscar II, King of the United Kingdoms of Norway and Sweden, presented the first ever Nobel Prize for Medicine or Physiology. The recipient was an immunologist, Emil von Behring, and he was being recognised for work that had led to the development of a new, life-saving treatment called antiserum.

As a medical researcher in the late 19th century, Behring was working in the golden era of bacteriology. In fact, he counted among his senior colleagues two bacteriologists who are still revered today as being among the greatest names in the history of modern medicine: Robert Koch and Paul Ehrlich. With supervisors like those, it would be hard not to win a Nobel Prize.

Behring's interests lay in applied science rather than basic research. He had done his medical training in the military where no doubt he would have become deeply aware of the horrors of wound sepsis on the battlefield. Entering research after leaving the military, he set his sights on important bacterial diseases afflicting the civilian population, especially diphtheria – a disease which at that time was among the commonest causes of death in children.

When Behring commenced his research, it was already known from animal experiments that the symptoms of diphtheria were caused by a toxin secreted by the bacterium responsible for transmitting the disease. Others then found that when guinea pigs were injected with this toxin in appropriate doses, they would develop immunity to its effects.

In work published in 1890, Behring took this a step further and showed that by taking blood from immunised animals and injecting the cell-free component of it into non-immunised animals, the latter would be protected from the effects of diphtheria toxin. This cell-free component of the blood collected from immunised animals became known as antiserum.

Probably most exciting of all for Behring, however, was that his studies showed that in addition to protecting healthy animals, antiserum could actually cure sick animals in which illness has been induced experimentally.

A year later, in 1891, Behring recorded the first use of antiserum in treating a human patient when a girl with severe diphtheria made a dramatic recovery.

By 1894, Behring had collaborated with a pharmaceutical company and production of antiserum was scaled up using large animals instead of laboratory rodents. Antiserum therapy expanded to the treatment of other toxin-related diseases and its success in the prevention of tetanus during WW1 earned Behring a military decoration. A century on, the sustained commercial success of antiserum production has kept Behring's name alive in the biopharmaceutical industry and in 2004 was brought closer to home following an acquisition by Australia's former Commonwealth Serum Laboratories to create CSL Behring. Coincidentally, the name of the monarch who presented Behring with his Nobel Prize has enjoyed a similar provenance – in 1902 he approved the use of his name and image by a Norwegian food company and we can still see him today in most supermarkets as the face of a familiar global brand of canned sardines.

It might be tempting to take the story of Behring and antiserum as an example of why the use of animals in research is ethically justified. Here we have a scientist conducting applied research to tackle a devastating clinical problem and through the use of a relatively small number of animals emerged triumphant in his quest to alleviate suffering. Behring's story could be added to that list of breakthroughs that pop up quite often in the animal research debate such as the discoveries of insulin and penicillin, the unmasking of the effects of thalidomide, and the development of organ transplantation.

But I think drawing simple links like these between research and longer-term outcomes runs the risk of oversimplification. Indeed, the fact that the same examples can, and often are, used by advocates on both sides of the argument shows that they are often selective and therefore biased.

I am therefore going to turn to another body of Nobel Prize-winning work, the impact of which has been every bit as great as that of Behring's, yet I think presents a far more realistic picture of how science really works. As such, it also presents a more useful platform from which to consider the challenges faced by Animal Ethics Committees (AEC) when trying to decide whether the potential benefits of a given research project are likely to justify the animal welfare costs.

In 1984, Georges Köhler and César Milstein shared the Nobel Prize for work they had done together in the 1970s. Like the work of Behring, their's sparked a medical revolution and proved to have enormous commercial application. Also like Behring, Köhler and Milstein had been working on that component of the immune system found in the cell-free part of the blood – the serum. But further comparison between these two bodies of work reveals a very important difference. Unlike Behring, Köhler and Milstein didn't set out to find a cure for something. They were not working in applied research, they were working in basic research and all they wanted to do was to solve an esoteric conundrum that had bothered the ivory-tower dwellers of immunology for decades.

So what was this great conundrum? It concerned that component of the immune system known as antibodies.

When an individual's immune system encounters something foreign – it might be a toxin as in the case of Behring's guinea pigs, or it might be a whole bacterium or virus – a central feature of the immune response involves the production of antibodies. These are Y-shaped proteins that bind to foreign invaders in a way that either neutralises them or triggers the deployment of other weapons in the immune system's arsenal.

The most remarkable feature of antibodies, though, is their specificity. That is, a given antibody is not able to bind to just any type of foreign invader; it can only bind to the specific invader to which it is matched. This feature has long been exploited by scientists who use antibodies as research tools and diagnostic reagents. In these situations, the antibody is labelled in some manner (most often a fluorescent tag these days) so it acts as a signal to indicate the presence of the foreign material to which it has bound.

What makes the specificity of antibodies so fascinating is that it requires the immune system to be able to generate enough different types of antibodies to make a specific one for any foreign invader an individual might encounter. This concept becomes even more stunning when we consider that in addition to being able to produce specific antibodies to the vast range of substances found in the natural environment, individuals will also produce antibodies to synthetic compounds that they would never have encountered throughout the entire evolutionary history of their species. The potential diversity of an individual's antibody repertoire is therefore almost

unimaginably huge – probably something in the order of 10^{12} different types.

The conundrum that occupied the minds of Köhler and Milstein, along with many other immunologists, therefore was how could the immune system generate such extraordinary diversity? How could it generate up to 10^{12} different types of antibody proteins? Some immunologists were even so awestruck by the idea of such a great yet mysterious process that they gave it a special acronym: the Generator of Diversity – GoD.

A range of hypotheses emerged to explain the conundrum of antibody diversity but it was eventually agreed the answer must have something to do with the genes that control antibody synthesis. By the 1960s – about 10 years before Köhler and Milstein began working together – it was known that each gene provided the code for one protein – the so-called one gene one protein principle.

This was an important advance in genetics, but it only made the immunologists' conundrum even more baffling. If there were something like 10^{12} different types of antibodies, the one gene one protein principle implied there must be a similar number of antibody genes to code for all those proteins. But even the most generous estimates of the total number of genes in a human or any other creature was nowhere near this – in fact, it was only about $1/20{,}000{,}000^{th}$ the number required if antibody synthesis was to follow the one gene one protein rule.

Clearly there must be something unique about the genes responsible for antibody synthesis which enabled them to create diversity in a way that no other genes in the body could.

Köhler and Milstein decided the best way to explore this conundrum was to analyse large numbers of known antibody genes in detail. But before they could do this, they needed to get over a technical obstacle. Isolating and analysing specific antibody genes from the vast mixture in normal animals or people was just too complicated. Even collecting cells from specially immunised animals wasn't good enough because the antibodies in antiserum are far from homogeneous – they all share the same target but they vary in affinity. These are known as polyclonal antibodies – 'poly' of course meaning 'many' – and even though they are useful in many applications, they aren't suitable for detailed study of the genes behind antibody synthesis.

Köhler and Milstein came up with an ingenious solution to overcome this obstacle by developing a method for making pure antibodies of any chosen type in unlimited quantities – these are known as monoclonal antibodies (MAbs).

Apart from being the perfect tool for their own experiments, Köhler and Milstein's technology for making MAbs offered other important benefits. For example, the final stage in the production process took place in pure cell culture. This meant the cell line for a specific antibody could be shipped easily between laboratories allowing scientists in one location to be sure their antibody was identical to the one their colleagues were using in another location, perhaps even on the other side of the world. This is not the case when using polyclonal antibodies isolated from the antiserum of immunised animals. And because monoclonal antibodies to a particular target are totally identical, they can also be exquisitely specific. This soon enabled the development of monoclonals for research and diagnostic tests that were far more sensitive and accurate than those which relied on polyclonal antibodies.

Köhler and Milstein never did answer the antibody diversity conundrum. But within a few years, their MAb technology had been adopted around the world and today monoclonals lie at the heart of countless research and practical applications including hormone assays, diagnostic tests, some new generation therapies, toxicology and even home pregnancy test kits. What's more, tests employing monoclonals have replaced many animal-based tests.

The development of monoclonals technology did require the sacrifice of many animals (mostly mice), and despite important refinements over the years, animals are often still required. Nevertheless, enormous benefits have flowed from this technology for both humans and animals and most would agree these benefits have outweighed the costs; the end has justified the means.

But what if we didn't have the advantage of nearly 40 years' hindsight? How might we have viewed this work from an ethical standpoint if it was presented to us as simply a research proposal with nothing other than speculation about how it might turn out?

I would like to pretend for a moment that we are an AEC in the early 1970s and we have been asked to review an application from Drs Köhler and

Milstein. Based on the published description of their experiments, I presented some ideas on the sort of discussion that might have taken place:

- The first thing that might have struck us is the technical complexity of the topic. With a title like "Continuous cultures of fused cells secreting antibody of predefined specificity", I would have to confess that I would have turned my attention to the other protocols on the agenda before steeling myself to trudge my way through this one. Some of the techniques they used were quite novel and even today, nearly 40 years on, their paper is not light reading. So unless it had been explained very clearly, it might have been difficult for us to understand the aims of the experiments in sufficient detail to be able to make an ethical assessment.
- Assuming we could actually make sense of the topic, it was very much within the realm of basic research. It doesn't set out to find a cure for something and the authors only made the vaguest prediction about its potential when they said the technique "could be valuable for medical and industrial use". Doing a prospective cost benefit analysis for basic research is always a challenge. Sure, it is sometimes possible to make a strong case in favour of basic research as the world has seen with the billions of dollars spent on the Large Hadron Collider. But would we as an AEC have found the case for unlocking the secrets of antibody diversity as compelling as the search for Higgs' boson?
- As I've pointed out, Köhler and Milstein's paper really only describes what was intended to be a means to an end: a tool they hoped would help solve the great antibody conundrum. But many other immunologists had already failed in this quest. Could we as an AEC justify the sacrifice of yet more animals in what might have seemed such an unpromising and esoteric field?
- And what about experimental design? Köhler and Milstein's paper makes no reference whatsoever to statistical analysis, nor does it mention blinded observation or randomisation. Should these have had a place in the study?
- Last but not least, we most certainly would have interrogated the application about the scope for alternatives. For example:
    - Their procedures used mouse cell lines. But even back then, it was possible to isolate the same type of cells from humans, so why couldn't these be used instead? What we might not have known is that only a year before, another group had attempted just that: to develop MAbs using human cells. But their attempts had failed and so did many subsequent attempts; it is only quite recently that techniques for producing monoclonals from human cells have become viable and even then they have limitations.
    - Could samples from humans have helped inform other aspects of their work? Yes – in fact they already had. A research hospital funded by the Rockefeller Foundation had conducted studies using blood from patients with a form of leukaemia known as myeloma. This cancer arises from the cells that produce antibodies so that patients have high concentrations of monoclonal antibodies in their blood produced by the cancer cells. This work (conducted by other researchers) yielded important information but, ethical considerations aside, there were sound technical reasons why it couldn't be applied any further by Köhler and Milstein.
    - What about using non-sentient organisms? Well, this is another technology that had already been exploited by other researchers but once again, it had reached its limit in relation to the study of antibody diversity.
    - Another 'alternative' we often hear advocated is the use of cell culture. Köhler and Milstein had in fact learnt this technique especially for their project – but again, technical constraints limited its application to only certain parts of the process.

Overall, I suspect our AEC would have concluded there was no real scope for alternatives. The question remains of course as to whether we would have been sufficiently convinced of the net ethical benefits to grant approval for the work to proceed.

Of course, this is all rather fanciful speculation. But it does show the complexity of how research is pieced together and that the appealing idea of a direct line between research and outcomes is too simplistic. It also shows the depth of understanding required by AECs if they are to make an informed judgment on whether the ethical cost of an animal experiment will ultimately justify the benefits, especially in the case of speculative, basic research. This is important because, at least according to some figures, basic research is

responsible for the greatest proportion of animals used in biomedicine.

Typical of its time, Köhler and Milstein's paper makes no reference to animal ethics approval so we will never know the outcome if it had been submitted to an AEC in the 1970s.

But there are hints to suggest others were not quite so convinced by the worthiness of this new technique at the time. Köhler and Milstein thought they were on to something pretty big so wrote up their findings in the form of a full length paper to *Nature*. The editors were not quite so impressed so asked them to re-submit the manuscript as a letter thus reducing its length by more than half. When issue 256 of *Nature* hit the news stands on 7 August 1975, it contained many letters from scientists in diverse fields. What is arguably one of the most important medical research papers in the later 20th century was well down the list at number 16.

But this pales when we consider the response to a proposal that MAb technology be protected through the UK's patenting authority. It was turned down on the basis that it was considered unlikely to have any great practical application. Today, the worldwide MAb industry is worth over $40bn.

If we as an AEC had had reservations about whether to approve Köhler and Milstein's application, we would at least have been in good company.

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Development of an animal well-being monitoring package

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Introduction

Effective monitoring of animal well-being has become a key metric to safeguard the welfare of animals used in research and teaching studies. In 2012, the University of Western Australia's Animal Ethics Committee (UWA AEC) instituted a requirement for all applications to include copies of the relevant animal monitoring sheets. However, UWA has a large portfolio of research and teaching projects, involving a wide variety of species and study locations. These range from traditional laboratory species (rodents and farm animals) to wildlife and aquatic field studies. Consequently, many different monitoring schemes were presented in a plethora of formats. They were often difficult to interpret by the range of stakeholders involved in animal care and the oversight of animal well-being. So a standardised UWA Animal Well-being Monitoring Package was developed and was recently endorsed by the UWA AEC.

Development of the UWA Well-being Monitoring Package

The aim was to provide a monitoring package that was pivotal to ensuring the welfare of the animals on *each* specific project and would accompany each AEC application. It was designed to:

- Provide a standard format that was easy to prepare and to interpret thus minimising the cognitive load for stakeholders.
- Be adaptable to a wide range of species and projects of varying complexity and invasiveness with options to select:
  - Relevant metrics of well-being.
  - Simple graded approach to assessment (non-binary).
  - Unambiguous pre-set decision points for intervention/action.
- Be flexible to update with new AEC policy.

Fundamentally, it also had to be consistent with the requirements of the Australian Code for the Care and Use of Animals for Scientific Purposes, 8th Edition, 2013 (The Code).

Code requirements

The Code defines monitoring as "measures undertaken to assess, or to ensure the assessment of, the well-being of animals in accordance with the Code. Monitoring occurs at different levels (including those of investigators, animal carers and animal ethics committees)". All stakeholders have their responsibilities defined in the Code.

Institutions are required to promote compliance by providing "adequate resources to ensure the AEC and people involved in the care and use of animals can meet their responsibilities, including monitoring animals". Institutions are also required to ensure that guidelines for the monitoring and assessment

of animals are "developed in consultation with the AEC, approved by the AEC, and implemented and promoted within the institution".

The Code also stipulates that the institution "must identify clear lines of responsibility, communication and accountability by ensuring that a person is responsible for the well-being of animals at any given time and is clearly identified". The Code expects "monitoring by competent people", and that the "scope of day-to-day monitoring must be clearly outlined and communicated to all parties".

The AEC must "monitor the care and use of animals, including housing conditions, practices and procedures involved in the care of animals in facilities" by "inspecting animals, animal housing and the conduct of procedures, and/or reviewing records and reports". The full extent of the AEC responsibilities is described in Section 2.3.17 to 2.3.23 of the Code.

Investigators are required to have "procedures in place for the monitoring and managing of animal health" from the planning stage of their project. In addition they:

- Must provide "details of how the well-being will be monitored and assessed throughout the project, the frequency of monitoring and assessment, the actions to be taken if problems are identified, and the criteria for intervention points and humane end-points".
- Are responsible for ensuring animal monitors are competent and "knowledgeable about the normal behaviour and signs of pain and distress for the species" or are "under the direct supervision of a competent person".
- Must "take steps at all times to safeguard the well-being of animals by avoiding or minimising known or potential causes of harm" by:
  - Ensuring "that animals are monitored and assessed at all stages of the project for signs of pain and distress, including deviations from normal behaviour".
  - Conducting assessments at "a frequency sufficient to detect such signs at an early stage, as determined by the procedure, and ensure that the planned end-points are detected".
  - Keeping records of monitoring and assessment of animal well-being.
  - Taking "prompt action" in accordance with "intervention points and humane end-points approved by the AEC".
  - Clearly outlining the scope of monitoring and "communicating to all parties" including animal carers.
  - Regularly reporting on "the monitoring of a new animal line at a frequency determined by the AEC".

Animal Facilities Managers and Animal Carers have the same responsibilities in terms of frequency of assessments, competency, record keeping, interventions and reporting to the investigator and the AEC.

More detailed information about monitoring methods, assessment criteria and actions, etc., are available in Section 3.1.21 to 3.1.28 of the Code.

Key components of the UWA Well-being Monitoring Package

The key components include:

- a cover sheet;
- a recording sheet; and
- additional sheets, e.g., anaesthesia monitoring sheets.

There is also a general instruction sheet to assist authors.

The cover sheet provides a framework which prompts the investigator to consider relevant animal welfare monitoring metrics, frequency of monitoring, scoring matrix, intervention and action points, as well as key personnel contact details and phenotype/model descriptions. The recording sheet delineates the planned schedule of monitoring for the chosen specific criteria and assessment score recording, in a uniform layout that is easy for all stakeholders to understand. Any current or forthcoming local AEC requirements can be readily incorporated. For example, if the AEC has set a specific limit for intervention and action, such as subcutaneous tumour size or body weight deficit action points, these can be added. These documents form the "monitoring paperwork" that must be submitted with a research or teaching application to the AEC for approval. Once approved, they will be kept with the animal in the relevant holding facility, as per UWA AEC requirements.

The package has now been in use at UWA for approximately 12 months. It works well and has had high acceptability by the research and teaching community, and animal-related support staff. As part

of the research support process at UWA, training and advice to applicants is available at regular workshops and on request.

Overall, the package facilitates easy design of animal well-being monitoring schemes, tailored to specific projects and species, which are flexible to enable incorporation of local AEC policy requirements and are consistent with the Code.

In the future we plan to undertake a formal survey of the various stakeholders to confirm that the package is meeting all of the desired aims. We also intend to incorporate additional features, such as drop-down menus, for easier selection of monitoring criteria and scoring system. We will also investigate the feasibility of incorporating the monitoring system into proprietary laboratory animal management software packages.

Engaging young adults in the issue of animal welfare

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Abstract

In 2011 ANZCCART New Zealand published a resource *Caring for the Animals We Use in Research and Teaching* designed to deepen 13- to 15-year-old students' understandings about animal welfare. In order to encourage older students to engage in this issue, practising teachers have developed new resources for 16- to 18-year-old students. These new resources are aligned with the National Certificate of Educational Achievement (NCEA) curriculum in Biology at Levels 1, 2 and 3, and utilise the ANZCCART resource. During this presentation examples of these new resources were shown and their potential for learning discussed.

Introduction

In 2011 the New Zealand Board of ANZCCART produced an education resource entitled *Caring for the Animals We Use in Research and Teaching*. This resource contains a DVD of video clips and a CD of educational resources to complement the DVD.

The DVD contains one compilation clip narrated by Dr Jessie Jacobsen (the 2007 MacDiarmid Young Scientist of the Year) and six clips where prominent New Zealand scientists talk about their research and how they use animals in their projects. It also has a clip where the legislation that governs the use of animals in research and teaching is discussed. To complement these clips, the CD contains resources that were written by experienced secondary teachers. These resources were written for 13- to 15-year-old students and focus on developing literacy associated with science and the nature of science (the way in which scientific knowledge is produced and verified). Two copies of the complete resource were sent to all secondary schools in New Zealand.

However, the clips in the DVD are also suitable for older students. Consequently ANZCCART decided to commission secondary teachers to write material for older students (16 to 18 years) based on the resource and to frame this new material around the senior secondary assessment framework.

NCEA and the new educational material

In New Zealand the assessment framework for educational attainment used to gain entrance into tertiary institutions is the National Certificate of Educational Achievement (NCEA) (see http://www.nzqa.govt.nz/qualifications-standards/qualifications/ncea/). NCEA comprises three levels of multiple achievement standards in each learning area, such as biology, physics, geography and mathematics. Each achievement standard is assigned a number of credits and students need to gain a set number of credits to gain an NCEA certificate at one of the three levels. Some achievement standards are assessed internally (within the school) and others are assessed through external examination.

The new material written to complement the DVD focuses on three of the biology achievement standards, one at each of Levels 1, 2 and 3. These three achievement standards are linked across the levels and are based on students developing an increasing understanding of the complexity of a scientific issue, in this instance the use of animals in research. The

three achievement standards that provided the focus for the material are:

Level 1 – AS 90926 Report on a biological issue (http://www.nzqa.govt.nz/ncea/assessment/search.do?query=Biology&view=all&level=01)

Level 2 – AS 91194 Analyse the biological validity of information presented (http://www.nzqa.govt.nz/ncea/assessment/search.do?query=Biology&view=all&level=02)

Level 3 – AS 91602 Integrate biological knowledge to develop an informed response to a socioscientific issue[1] (http://www.nzqa.govt.nz/ncea/assessment/search.do?query=Biology&view=all&level=03)

Material content

When writing the material each writer was asked to develop a 'ready to use package' that other secondary teachers would be able to access and use in their classrooms. Each package contains:

1. A copy of the achievement standard downloaded from the New Zealand Qualifications Authority (NZQA) website. This is a generic statement that provides information for teachers that explains how to contextualise the standard as well as the requirements for the levels of achievement within the standard (Achievement, Achievement with Merit, Achievement with Excellence). Information such as the link between the standard and *The New Zealand Curriculum* (Ministry of Education 2007) along with definitions of the terminology used in the standard are provided.
2. A copy of the conditions of assessment downloaded from the NZQA website. This provides teachers with more information about specific requirements for each achievement standard, for example the number of situations that students need to address and the number of re-assessment opportunities that should be provided.
3. Planning notes for teachers that contextualise each achievement standard within the issue of the use of animals in research.
4. The assessment task(s), the marking schedule, along with the assessment criteria for an Achievement, Achievement with Merit, and Achievement with Excellence in the standard.
5. A range of resources to accompany the planning notes that provide information about the issue from multiple viewpoints, for example ANZCCART's *Animal Research Saves Lives*, media articles, links to video clips and the recent SAFE publication *Animals in Science: Ethical Arguments and Alternatives to Animal Experiments*.

Once the packages have been completed, they will be checked for scientific accuracy and copyright and then sent to the NZQA for 'qaaming'. 'Qaaming' means that each package will be moderated by NZQA experts so that it is approved by them as meeting the requirements for the nominated standard. Next, information about the packages will be disseminated by ANZCCART through science subject associations and the ANZCCART websites. Secondary teachers wanting to teach a particular standard in the context of the use of animals in research will then be able to contact ANZCCART and request a copy.

Specific requirements for each standard

AS 90926 Report on a biological issue (Level 1)

In order to achieve this standard, students need to write a report. This report is to be about the biological issue[2] of the use of animals in research. It is to be written after 2-3 weeks studying multiple viewpoints. In the report students are expected to show evidence of:

- having refined a given question and/or purpose related to the issue;
- being able to describe biological ideas that have been presented to the students from a range of resources;
- having collected and/or processed data, for example evidence of use of summarising; and
- being able to present findings and take a position on the issue.

This report is written in open-book exam-like conditions.

[1] A socioscientific issue is an open-ended, controversial issue that is science-based but also has social, ethical, moral and political dimensions. This type of issue is current and relevant so students work with situations that are real. Such issues often have local, national and global dimensions. Also these issues often have multiple possible solutions and when making a decision about a possible solution, an understanding of risk and probability can be needed.

[2] A biological issue is defined by the NZQA as one where people hold differing points of view.

AS 91194 Analyse the biological validity of information presented (Level 2)

The teaching associated with this standard involves students examining and identifying biological features involved in an issue, as well as identifying bias, the purpose of information, who has a vested interest in that information, and examining how such information would affect the public.

This standard is then assessed by students analysing three different information sources about the issue of the use of animals in research (for example, a video clip, a newspaper/magazine article, a scientific paper) on three separate occasions. In their analysis students need to be able to:

- identify biological features in the information source supplied;
- identify information as accurate/inaccurate/biased using biological knowledge about the issue;
- correct any inaccuracies found within the information source; and
- identify the purpose of the information source, for example the intended audience.

Like AS 90926, this assessment is carried out in exam-like conditions.

AS 91602 Integrate biological knowledge to develop an informed response to a socioscientific issue (Level 3)

At Level 3 (Year 13), students are expected to explore the issue of the use of animals in research independently with guidance from their teacher. Students have to construct a presentation, for example a report or a poster, that presents and justifies their personal viewpoint about the issue, and propose action that could be taken. This action can be either individual or collective. A presentation needs to:

- explain the position the student has taken and why an action has been chosen;
- contain relevant biological knowledge about the issue; and
- evaluate the biological knowledge used, for example commenting on its validity and bias, comparing significance of implications, and considering the effectiveness of actions.

In conclusion, by disseminating these resources, it is hoped that more students will have the opportunity to develop an informed position about the issue of the use of animals in research and teaching.

Surviving the External Review: a user's perspective

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Abstract

External reviews of Animal Ethics Committees (AECs) and institutional processes have become an accepted and valued part of the regulatory process which underpins the work of AECs. While the review panel tends to focus on the effectiveness of the AEC and the committee's impact within its institution, there is often much more an AEC can learn throughout the process. In our institution we have, over the past 7+ years, developed an anonymous survey which is distributed to all those who have applied for AEC approval during the period under review. The results of the survey provide important insights to the review panel and a baseline to use as part of the evaluation of other interactions across the institution. More importantly, the results provide insights to the members and officers of the AEC. Based on a much wider proportion of AEC applicants than the external review interacts with, it is possible to identify areas of satisfaction and areas which can be improved, it is possible to ask specific questions of interest and, above all, the data can be compared from one review cycle to the next providing an indication of trends. In this presentation I highlighted the survey tool we have developed, described how we use it, reflected on its value, and identified how the insights gained from it have been applied more widely.

Since 2006, the idea of external review of Animal Ethics Committees (AECs) – at least in Australia – has become a mainstay of the animal ethics process. In the early years, it is probably fair to say that there was some resistance to the idea. More recently, positive acceptance has become the norm, and in most instances institutions have acknowledged the value of the reviews conducted.

Our institution's AEC has now been externally reviewed on three occasions (2007, 2010 and 2013). Before the first of these reviews, we thought about how it would be possible to provide an external panel with a range of insights into the institution, the range of AEC applications, and the attitudes and culture of the people associated at all levels with the processes. While the panel interviews a number of people, it is not possible for everyone to be interviewed. In some instances, we felt that particular perspectives were being represented to the panel and it could be useful for them to understand more fully the overarching context out of which those views had emerged.

Rather than providing the panel with extensive briefings, which may have resulted (even inadvertently) in influencing or even compromising some of their independent advice, we decided to conduct a short anonymous survey of all people who had any interaction with the AEC during the triennium. A survey was designed by the Ethics Office in consultation with survey experts and was conducted through an administrative area, at arm's length from the AEC. The resulting questionnaire, utilising the QUALTRICS technology, was sent to all staff and students involved in the use of animals. For the 2010–2012 triennium the survey was sent to 318 individuals and 104 responses were received.

The survey was designed with 32 questions where respondents were asked to select a response from a 'pick list' of options. In some instances respondents found that the options provided did not entirely fit their needs, for which most questions included a category of 'other' with an opportunity to provide a brief explanation.

Each question also offered respondents an opportunity to amplify their selection with comments. These comments were later provided to the review panel in an unedited format so that the panellists were able to see all comments exactly as they had been made against each question.

QUALTRICS enabled us to present the data for each question both as a table and pie chart, as well as to cross-tabulate particular questions (e.g., linking a respondent's level of appointment with the number or type of applications they had submitted, or listing all comments according to level of appointment or number of applications submitted). However, we took care as far as possible to provide the data in an un-interpreted fashion to enable the panel to gain insights and draw their own conclusions.

We asked questions in many areas. We asked questions about a person's position or level of appointment; their level of involvement in animal research; the number of applications each person had submitted to the AEC during the triennium; and the types of animals they mainly work with. We asked individuals to rate their levels of understanding of and confidence with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

We asked about their overall rating of the Ethics Office's levels of service and provision of advice. We asked specifically about the value of information available on our website and in our newsletter as well as training and registration information. We asked whether they had received a monitoring visit from the Animal Welfare Officer and how useful that interaction was. We asked about the AEC's reputation, the ease of use of application forms, and the committee's communications and processes in response to applications: timeliness, clarity and helpfulness. We asked people to rate the overall process and think about priorities for the next triennium. Comments and suggestions were welcomed, and people were given an opportunity to identify themselves and ask for a meeting with the review panel (which was promptly separated from their survey content).

The results of the survey have been astounding. Given the relatively high response rate, we are confident that a range of both positive and more critical views are well represented. The overall range of responses was representative of the range of AEC applicants. From our perspective, some of the most powerful aspects of the results were the comments. While there were some of the 'usual' criticisms which many AECs hear, and some fair criticisms of our committee and its processes, these have led us to reconsider the elements criticised and to make improvements or clarify communication where possible. As we were aware of the appointment level of each person who made comments, we have been able to plan more targeted communication and education for specific categories or groups of staff. The most empowering element of the feedback was the comments made concerning the strength and willingness of the staff in responding to applicants, the significant levels of service provided, and the ongoing improvements which had been reached. It was also interesting to note that even where staff felt that the AEC decision may have been somewhat harsh or may have misunderstood the project, most individuals felt comfortable approaching the office for advice and assistance in addressing the issues raised. Particularly noteworthy were several comments reflecting on the Animal Ethics Officer or the Animal Welfare Officer providing clear advice on complex or unique issues. As a whole the exercise reaffirmed and enabled us to celebrate that we are doing a good job, it provided wider evidence than the panel would ordinarily access concerning people's opinions and it provided us with some clear guidance concerning improvements which can be made. It also showed that the AEC is making an institution-wide impact in many areas related to animal welfare. The abiding learning has been from fair criticisms as well as constructive suggestions and accolades where warranted.

These results have added power to the review itself and beyond this have given the AEC and the university data on which to base judgments and decisions. We will continue to develop this survey and utilise a further updated version at our next External Triennial Review.

Training and support for Animal Ethics Committee members

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Abstract

The Code of Ethical Conduct (CEC) under which a New Zealand Animal Ethics Committee (AEC) operates undertakes to provide new members with a National Animal Ethics Advisory Committee (NAEAC) new members' induction pack as part of their formal introduction to the committee. Following this, many committees also provide additional training including attending the biennial NAEAC workshops and annual ANZCCART conferences. However, the type and amount of training that committee members receive can vary greatly, and different committees may require specific training depending on the type of applications they consider, meaning that "one size does not fit all". Formal assessment of training in committees is currently carried out during their five-yearly audit. To date, New Zealand AEC members' training requirements have not been surveyed. This presentation focused on the results of a survey of members to assess the level of training and support that is provided to them; and to find out what we do well, and whether there are areas where we can make improvements.

Introduction

I would like to start by acknowledging the survey respondents, and to thank them for donating their time to make the replies. Without their participation, I would not have been able to make this presentation! Also I would like to thank colleagues who encouraged me and critiqued the survey, and the New Zealand Ethics Committee (NZEC) for reviewing my application and giving me Human Ethics Approval.

What is support? The dictionary definition says "bear all or part of the weight of, hold up". I considered support to be all round, and, in keeping with the ethical and welfare framework for the ANZCCART Conference, I used the Five Domains concept (Mellor & Reid 1994), i.e., the physical or functional domains of: 1. Nutrition; 2. Environment; 3. Health; 4. Behaviour; and 5. the neurological domain of Mental State and feelings, or in this case provision of training to aid the mental state.

The New Zealand Animal Welfare Act 1999 makes provision for the use of animals in Research, Testing and Teaching under Section 6. This allows for the use of animals for this purpose through organisations having a CEC (Code of Ethical Conduct) and an Animal Ethics Committee (AEC) with a prescribed membership. NAEAC (National Animal Ethics Advisory Committee) is the governing body, administered through the Ministry for Primary Industries (MPI), that looks after the AECs and reports to the Minister of Agriculture. In 2014 we have 30 AECs with a minimum of four members per committee. The membership may be internal or institutional members (from within the organisation to which the CEC belongs) or independent members from outside the organisation.

The survey aim was to find out what training and support these committee members received. Out of a possible minimum 120 and likely maximum 150 members, we had 82 survey responses which was a 55-68% response from the membership. Research shows that for 'in house' surveys there is often a 30-40% response rate (Survey Gizmo) so this level of response was extremely encouraging. The potential of

risk for participants (revealing information sourced from them) when using survey information in a public forum meant that human ethics approval was required for the survey and for use of the data. This was applied for from the New Zealand Ethics Committee (NZEC) which is a charitable organisation providing ethics approval for researchers and/or organisations in New Zealand who do not have an affiliation with hospital and university human ethics committees. The application was entitled: "Training and support for Animal Ethics Committee members in New Zealand" and was given an approval number NZ Ethics 13 14.

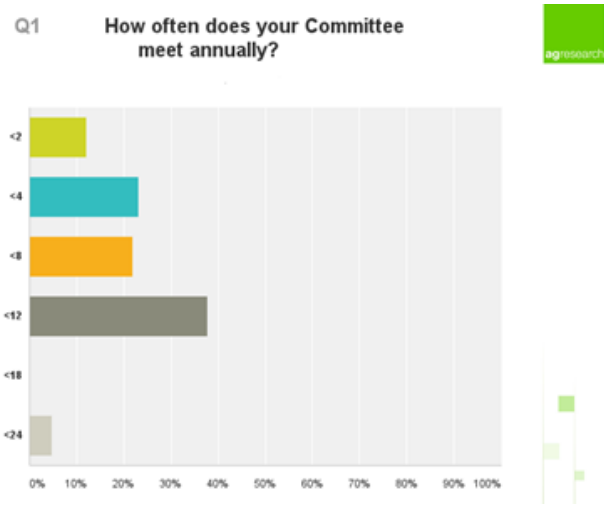
In New Zealand, there is a formal induction pack given to members. This pack is now available in an electronic form which is available from MPI. This comes from the supervisory body, NAEAC, and contains the following documents:

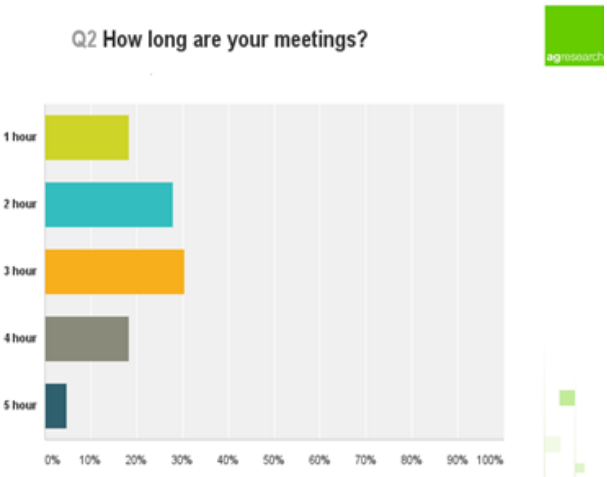
- Letter from the chair person;
- The Use of Animals in Research, Testing and Teaching – Users' Guide to Part 6 of the Animal Welfare Act 1999;
- A Culture of Care – A Guide for People Working with Animals in Research, Testing and Teaching;
- Good Practice Guide for the Use of Animals in Research, Testing and Teaching;
- NAEAC Occasional Papers 1-10;
- Animal Use Statistics;
- Latest issue of the NAEAC Annual Report;
- Animal research has benefits for us all – and for animals too (available for download from *Understanding Animal Research*);
- The Three Rs: past, present and future (UFAW);
- The role and evolution of independent Government advisory committees: the New Zealand experience from 1985 to 2005; and
- A Guide for Lay Members of Animal Ethics Committees.

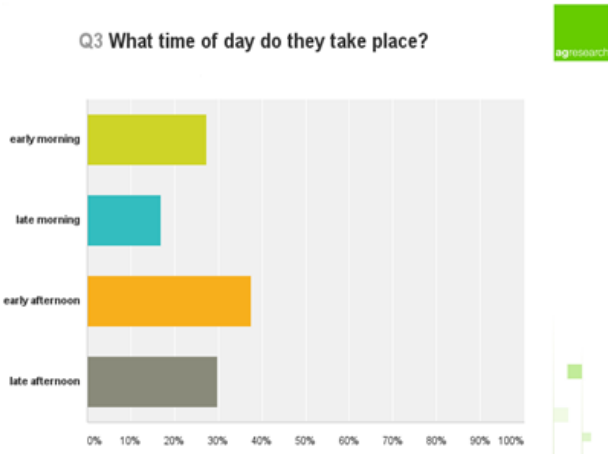
Dr Mark Fisher in his presentation at the ANZCCART Conference 2014 discussed background reading for ethics and welfare and asked a pertinent question "who has read all these?" (Fisher et al. 2014). In my experience these documents are useful, but they are not light bedtime reading, and if you do not understand the background to the written material, they can be meaningless.

In this presentation, the survey questions and answers (made through Survey Monkey) are presented with no analysis of the membership making the replies. This was not the remit for the current presentation, but further analysis of the results is planned, possibly using Qualtrics, as advised by Erich von Dietz (von Dietz 2014).

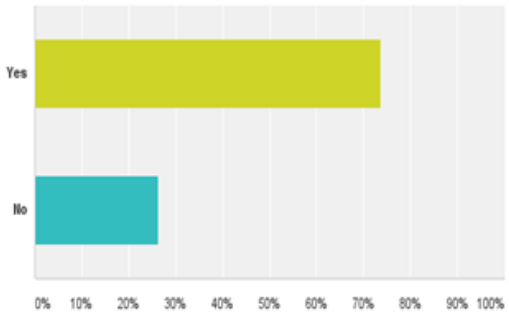
Survey responses



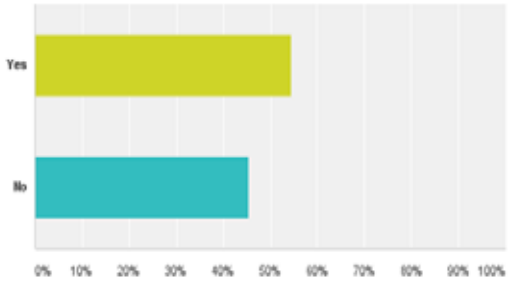




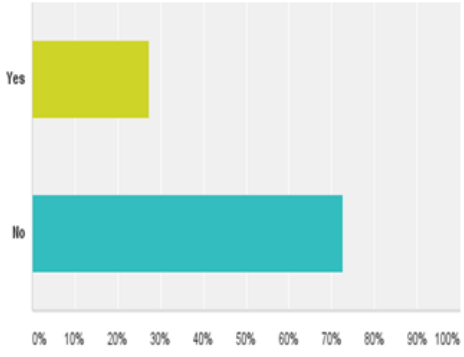
Q4 Do you receive refreshments?



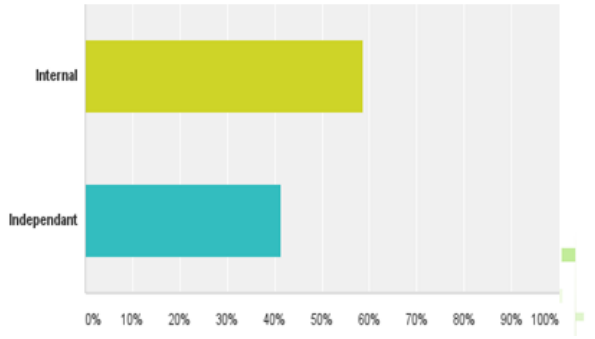
Q5 Do you receive remuneration for the time (both preparation and meetings) involved?



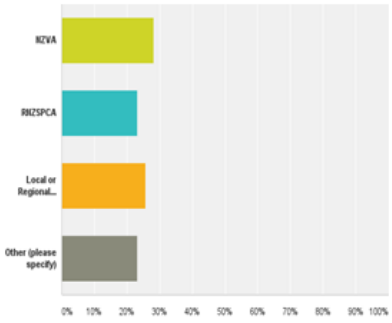
Q6: Do you receive reimbursement for parking, travel?



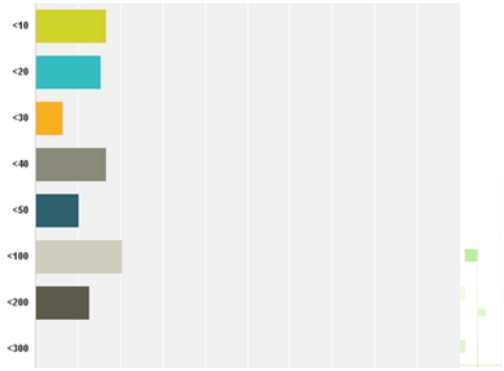
Q7: Are you an **internal** or **independent** member of your ethics committee?



Q8: If an independent member, are you nominated by the NZVA, the RNZSPCA, a local or regional council, or some other body?

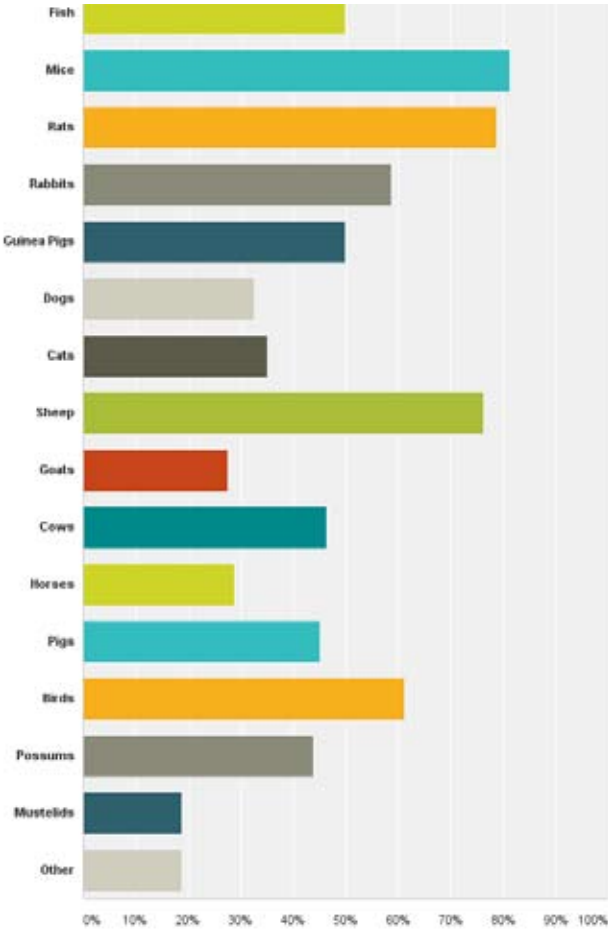


Q9: How many applications do you view annually?

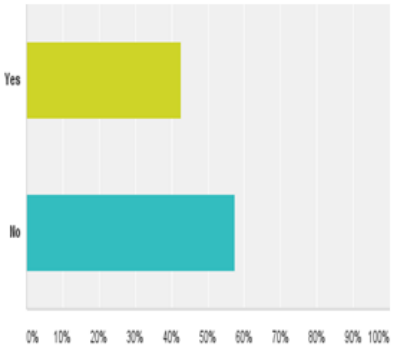


Q10: What range of species do your applications cover?

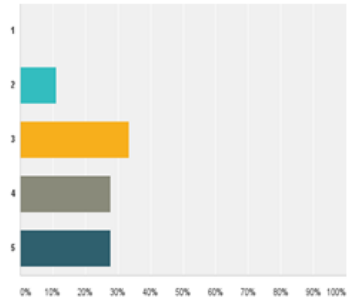
- Terrestrial Mammalian species and
- Crabs
- Lizards
- Frogs
- Terrapins
- Octopus
- Marine mammals
- Fish
- Birds



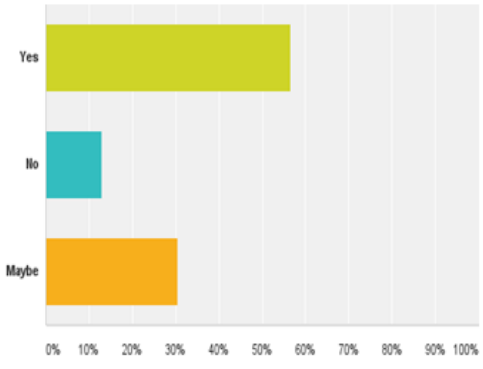
Q11: Did you receive a formal induction when you started as a Committee member?



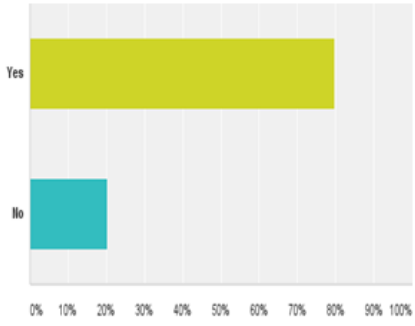
Q12: If Yes to 11. Rate usefulness and relevance of induction from 1(poor) to 5 (excellent)



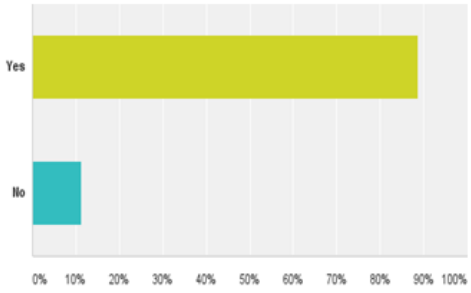
Q13: Did the induction package prepare you well for the material covered in meetings??



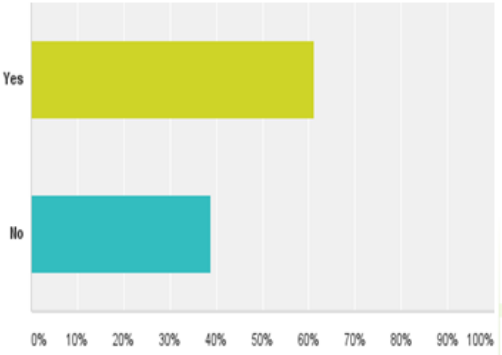
Q14: Did you have any background experience in Animal Welfare or Research?



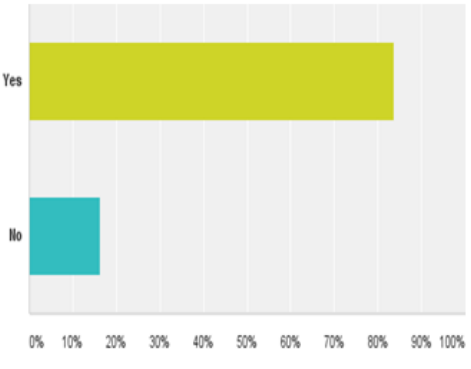
Q15: Do you have an opportunity for informal discussion at your meetings? Or on occasions separate to the official committee meetings?



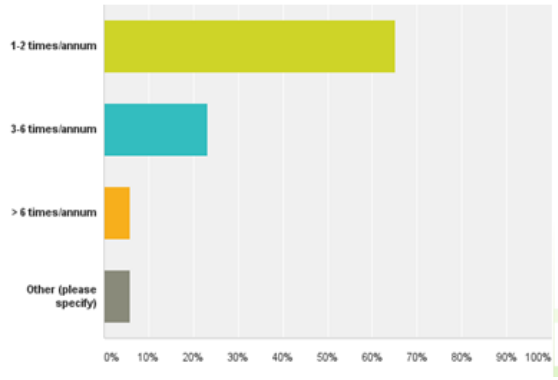
Q16: Do you invite experimental proposal applicants to speak at your meetings?



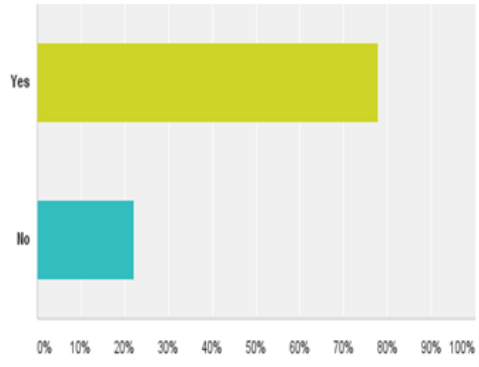
Q17: Do you make visits as a committee to projects and facilities?



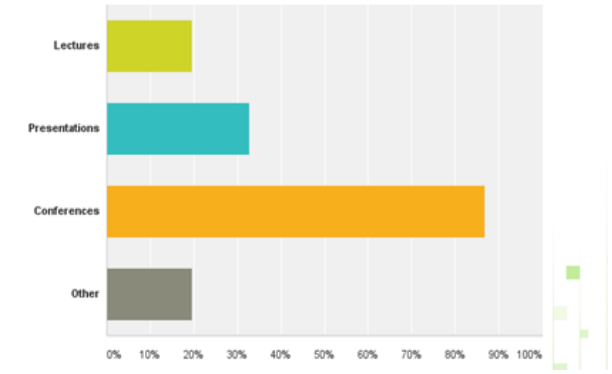
Q18: If Yes to 17: How often do you make visits?



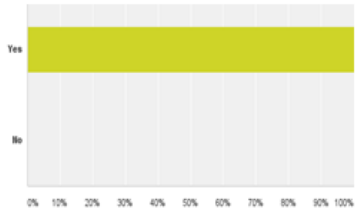
Q19: Continuing Education – are you provided with opportunities?



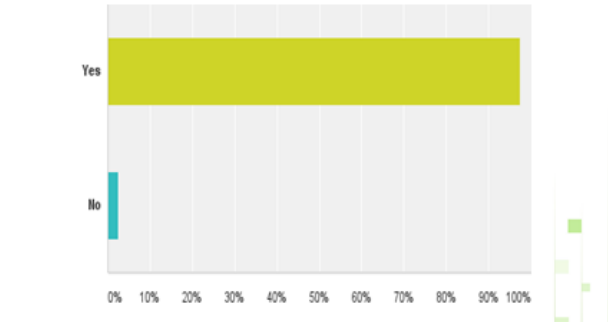
Q20: If yes to 19. What type of education?



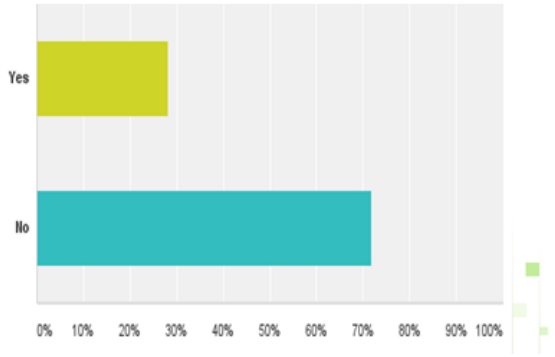
Q23: Do you enjoy your Committee Work?



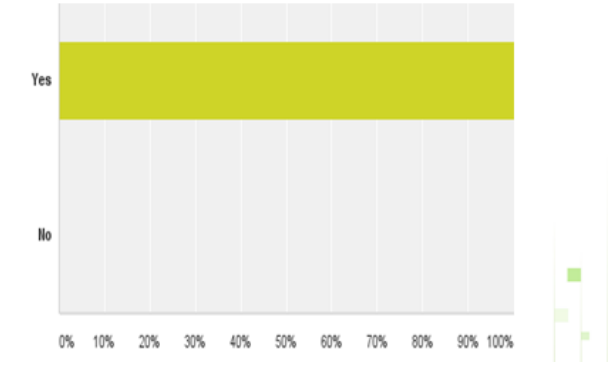
Q21: If you do not feel comfortable with an experimental proposal, do you feel you have adequate avenues available to obtain relevant information to make a good decision?



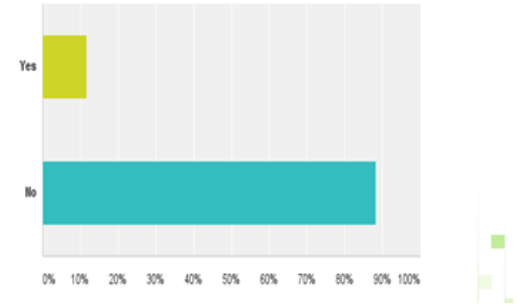
Q24: Do you have suggestions for improvement of support and training available to Animal Ethics Committee?



Q22: If you have concerns with a proposal, do you feel they are heard and considered by your fellow committee members?



Q25: Do you have any points not addressed in this survey that you would like to be considered?



Discussion

The replies to the questions show considerable variation in the degree of training and the level of functional support received. This is in keeping with the findings of Burnett & Zurawski (2014). The committees vary widely in the number of applications they review (10 to up to 200 per annum) and the number of species covered in those applications (see Question 10). Less than 50% of responding members said that they received a formal induction when they started as a Committee member. However, many of the respondents who received no training were founder members of an AEC, which was before formal training was recognised as necessary or advantageous. 80% of respondents said that they had some background in animal welfare or research. Of those who received an Induction Pack, 89% rated its usefulness at a score of 3 or greater (score 1 poor to 5 excellent), and 56% felt that the pack prepared them well for the material covered in meetings.

More than 70% of respondents felt that the welfare domain of nutrition was provided for! Some meetings were short (one hour) and did not require refreshments. However, the comments indicated that for some members, improvement in this type of support would be helpful. The domain of environment was not examined by the survey, and perhaps this should have been assessed by devoting some questions to the suitability of meeting rooms and the provision of parking. The physical domain of health covers remuneration for time and parking, and these were provided to 54% and 28% of respondents, respectively. Comments from internal members indicated that the meetings were part of their full-time job description; therefore they responded "No" to remuneration. However, some members donated their time to the AEC work.

The domain of behaviour was considered when members responded to Questions 21 and 22. 100% of respondents felt that their concerns were heard in meetings, and 98% felt that they had adequate avenues to seek information about proposals to enable decision-making. The comments from those who did not implied that training for all members of the committee and researchers was important to ensure that everyone was appraised of current welfare science and thinking.

The fifth mental domain was peripherally addressed in many of the questions. The fact that 100% of respondents said they enjoyed their AEC work suggests that most respondents felt this was a good domain. However, some qualified the word "enjoy" to mean that their input was worthwhile and fulfilling rather than being true enjoyment. Many respondents felt that additional avenues for training, especially online forums, study modules and webinars, would be helpful. Also opportunities to attend the meetings of other committees, and discussion between members of other committees, would aid members to make balanced ethical and welfare assessments of research proposals.

Conclusion

The survey results highlighted the fact that whilst there is provision for training and support to Animal Ethics Committee members, the actual level of training and support received by individuals is extremely variable. The comment that members do not know how to make a true ethical analysis of a proposal has been a criticism levelled at Animal Ethics Committees, and this may partly reflect the training provided to members. The survey indicates that further training resources for Animal Ethics Committee members would be appropriate, in keeping with the findings by Ideland (2009).

Acknowledgments

Thank you to ANZCCART for the invitation to make the presentation.

Also thank you to John Schofield; Virginia Williams; Jim Webster and Ruakura AEC members; Martin Tolich and NZEC; Paula Lemow; Linda Carsons; Secretaries and Members of NZ Animal Ethics Committees for help with compiling, distributing and responding to the survey.

References

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Fisher, M.; Diesch, T.; Orange, M. 2014: Scapegoats and tourists – science as the source of, and the answer to, ethical dilemmas. Pp. 1-7 *in*: Proceedings ANZCCART Conference 2014.

Ideland, M. 2009: Research ethics. Different views on ethics: how animal ethics is situated in a committee culture. *Journal of Medical Ethics 35*: 258-261.

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Oppermann, M. 1995: Email surveys – potential and pitfalls. *Marketing Research 7*: 28-33.

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Links

Animal Welfare Act 1999, Section 6: http://www.biosecurity.govt.nz/regs/animal-welfare/pubs/animal-welfare-act-1999 (accessed 12.11.2014)

New Zealand Ethics Committee (NZEC): www.nzethics.com (accessed 12.11.2014)

Survey Gizmo: http://www.surveygizmo.com/survey-blog/survey-response-rates/ (accessed 12.11.2014)

Survey Monkey: https://www.surveymonkey.com/ (accessed 12.11.2014)

Cam Reid Oration 2014

How being nice to mice made my research career

Dr Siouxsie Wiles

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Auckland, New Zealand
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A tale of two passions

Many people are surprised when they learn that, as a teenager, I objected to the rat dissection we had to carry out during biology class. While the rest of my classmates were getting to grips with the finer points of rat anatomy, I was investigating the production of cyanide by buttercups. So how then did I become the only one of my class to go on to become a researcher using animals?

My story begins with two books. The first is "The Fireside Book of Deadly Diseases" by psychiatrist Robert Wilkins, a treasure trove of facts and anecdotes that detail the influence of infectious diseases on both people and the course of history (Wilkins 1994). The second is "The Hot Zone" by Richard Preston, which describes the discovery of the Reston strain of the Ebola virus in a primate quarantine facility in Reston, Virginia, a stone's throw from Washington DC (Preston 1994). It was 1994 and I was in my first year at the University of Edinburgh studying biology. I had initially thought that I would specialise in genetics, but those two books changed everything. I was fascinated by the tales of deadly microbes, of how some bacteria and viruses are able to kill a healthy person, sometimes in just a few days. I ditched genetics and ended up specialising in medical microbiology.

After Edinburgh, I took a slight detour from medical microbiology to pursue another passion, that of bioluminescence, the production of light by living creatures. This simple chemical reaction allows fireflies to find a mate, anglerfish and glow worms to find food, and nocturnal squid to camouflage themselves while hunting. One of my favourite bioluminescent creatures is a soil bacterium called *Photorhabdus luminescens* which forms a deadly partnership with a tiny parasitic worm called a nematode (Waterfield et al. 2009). The nematode houses *P. luminescens* in its gut and has a nasty habit of burrowing itself into the larvae of various insects. Once inside a larva, the nematode regurgitates *P. luminescens*, which starts to produce toxins, killing the larva. The decaying larva provides an excellent supply of nutrients for the nematode and bacteria to feast on as they reproduce. In fact, decaying larvae produce such a great supply of nutrients that *P. luminescens* also produces antibiotics, to kill other microbes that may try to take advantage of the situation. Once the nematodes and bacteria have reproduced, the nematodes will swallow some *P. luminescens* once again and then spill out of the dead larva, looking for their next victim. *P. luminescens* also glows, lighting up the little insect corpse like an eerie Christmas tree decoration. It's thought the light might help to attract other larvae, providing a supply of fresh prey. As an interesting footnote, it has been reported that during the American Civil War some injured soldiers had glowing wounds, and that these soldiers were much more likely to survive their injuries. The phenomenon became known as Angel's Glow. However, there is nothing supernatural about it. A much more likely explanation is that the soldiers'

wounds had become contaminated with *P. luminescens* from the surrounding soil. The antibiotics produced by *P. luminescens* would have stopped other nastier bacteria from colonising the soldiers' wounds.

From wastewater to guts!

Almost 25 years ago, researchers identified the genes that make *P. luminescens* luminescent (Frackman et al. 1990), allowing scientists to engineer non-luminescent bacteria that glow. There are a number of reasons why this is useful. For example, light can be used as a surrogate for measuring the number of bacteria present in a sample, instead of waiting for the bacteria to physically grow on petri dishes which can take weeks or even months for some microbes. The more bacteria there are, the brighter the light. Also useful is that only living cells glow as the chemical reaction requires energy; if the bacteria are killed their light goes out.

During my PhD studies I used bioluminescence to develop 'biosensors' for monitoring the health of microbes responsible for carrying out remediation in an industrial wastewater treatment plant. We isolated, and then made luminescent, a number of these 'wastewater' bacteria and generated a suite of sensors that could be used to assess the toxicity of the wastewater entering the remediation system, all in just a few minutes (Wiles et al. 2003, 2005b). While enjoyable, a PhD in environmental microbiology was enough to make me realise that infectious microbes were where my interests really lay, so for my first postdoctoral position I moved to Imperial College London to work with a group studying how *Mycobacterium tuberculosis* causes the lung disease tuberculosis (TB). It was here that I took my first steps towards using animals in my research, and was introduced to the Three Rs ethical framework first described by William Russell and Rex Birch (Russell & Birch 1959): the use of non-animal methods to achieve the same scientific goals (*replacement*) or, where this is not possible, that researchers use methods which cause the minimum pain and suffering (*refinement*) while also obtaining the best information possible from the fewest number of animals (*reduction*).

The use of animals still plays an important role in many aspects of infectious diseases research. This is partly because of the ethical problems of exposing humans to potentially lethal agents, but also because it is currently very difficult to reproduce the dynamics of many infectious diseases without both the infectious microbe and a susceptible host. It is in this area that bioluminescence really shines. One of the most amazing things about light is that it travels through flesh and skin. You can see this if you put your hand in front of a torch. Traditionally, infected animals are euthanised at defined time points and tissues are removed for determination of microbial numbers after homogenisation and incubation on selective agar. In contrast, the technique of biophotonic imaging uses sensitive cameras to visualise light from within living animals (Andreu et al. 2011). This allows researchers to detect where their light-emitting cells are, how many there are and how this changes over time – all without having to euthanise the animals. Biophotonic imaging therefore massively reduces the numbers of animals needed for experiments and means researchers do not have to rely on animals showing any physical signs of disease.

Whilst working at Imperial College, I made a bioluminescent strain of *Citrobacter rodentium* (Wiles et al. 2004b), a bacterium that infects laboratory mice in the same way some strains of food poisoning *Escherichia coli* infect humans (Mundy et al. 2005). Giving mice food poisoning usually involves delivering a dose of bacteria directly into the animal's gastrointestinal tract via oral gavage. In my initial experiments, this was just what I did and I found that the first part of a mouse that becomes colonised by *C. rodentium* is the caecal patch (Wiles et al. 2004, 2006; Mundy et al. 2005), a group of immune cells that are the equivalent of our appendix. This was an interesting finding, but I wanted to look at the natural spread of disease rather than using artificial methods of infecting the mice. I designed an experiment involving one artificially infected mouse living with uninfected mice and used biophotonic imaging to track the bioluminescent *C. rodentium* as it spread from mouse to mouse (Wiles et al. 2005a). I was excited to discover an important difference between the artificial and natural infections. Bacteria that are shed in the faeces of infected mice are 'hyper-infectious'; they go on to infect other mice at a dose that is 1,000 times higher than bacteria grown in artificial laboratory media, are readily transmitted from infected to naïve animals and infect different niches within the gastrointestinal tract (Wiles et al. 2005a). This simple experiment led to a major refinement of the *C. rodentium* infection model,

making a more realistic model for human disease while requiring fewer animals to undergo the more invasive oral gavage procedure. In 2005, a vet who I worked closely with at Imperial College suggested that I apply for the inaugural 3Rs award that had just been announced by the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), which I went on to win.

This simple finding has also opened up a new avenue of research for my laboratory. Bacteria are able to easily change their genetic material; they can mutate and rearrange their genes, or even pick up new genes from their surroundings. This adaptation is how bacteria become resistant to antibiotics and how new diseases can seem to appear out of nowhere. For example, a few years ago Germany was in turmoil when an unusual strain of *E. coli* caused an outbreak of food poisoning with an unusually high rate of kidney failure (Jandhyala et al. 2013). Over two months, nearly 4,000 people were affected and 54 people died while officials tried to find the source of the outbreak. Was it lettuce, or cucumbers? It turned out to be beansprouts. Somewhere in northern Africa a strain of *E. coli* picked up a gene for a toxin from another closely related *E. coli* strain and then contaminated a consignment of bean sprout seeds destined for Europe. But how did this new strain come about? How did it evolve? We are investigating these questions using our *C. rodentium* natural transmission model. Over many months we have been allowing *C. rodentium* to transmit from mouse to mouse, freezing bacteria from each infected animal to give us the bacterial equivalent of a fossil record. What we want to know is will *C. rodentium*, over time, become more infectious or less? Will the bacteria adapt to infect new niches within the mouse? We'll soon find out.

Expect the unexpected

Being able to visualise what bioluminescently labelled bacteria are doing in real-time during an infection has the potential to challenge conventional dogma and open up new research avenues. We have recently developed a mouse model for nasal/throat carriage of the bacterium *Streptococcus pyogenes* to test potential vaccines (Alam et al. 2013a). Almost everyone will have had an *S. pyogenes* infection at some stage in their life – it causes tonsillitis – and many people will be unknowingly carrying the bacterium in their throats at this very moment. *S. pyogenes* can also cause a life-threatening illness called necrotising fasciitis, literally the flesh-eating disease where the bacterium produces an enzyme that digests tissue, which can require amputation of the infected limb. By inoculating the noses of mice with bioluminescent *S. pyogenes*, we discovered that some animals went on to get glowing vaginas (Alam et al. 2013b). This finding was an excellent demonstration of the flexibility of *S. pyogenes*, that diseases can take unexpected turns, and that biophotonic imaging can help us spot things we would have otherwise missed.

Replacing mice in the search for new antibiotics

Infectious microbes are responsible for one in every four deaths worldwide (World Health Organisation 2011). Here in New Zealand, one in every four people who are admitted to hospital overnight are there because of an infectious microbe (Baker et al. 2012). Our rates of many infectious diseases are higher than countries like the United Kingdom and Australia, and are rising (Williamson et al. 2013a,b,c; Williamson & Heffernan 2014). It is also clear that we have been living in the golden age of antibiotics, an era which will soon be over. A 2014 report by the World Health Organisation examines the consequences of our overuse and misuse of antibiotics in farming, and human and veterinary medicine (World Health Organisation 2014), and highlights what microbiologists have been shouting for a while now: a world without antibiotics is a scary place, and we are likely to be living in that world in as little as ten years. One of the most useful features of bioluminescence is that only living cells glow – the chemical reaction needs energy so if the bacteria are killed, the lights go out. This means we can use glowing bacteria to try to find new antibiotics – we just add the bacteria and experimental medicines together and then look for light. We can get an answer in just a few hours, or even minutes. This means using bioluminescence can massively speed up the discovery and testing of new antibiotics, which the world desperately needs.

To help us in our search for new antibiotics, we are developing bioluminescent strains of many different bacteria. It is here that we have made one very simple change to how we determine if our bioluminescently

labelled bacteria produce enough light to be detected from within an animal. The standard method for doing this is to inject different dilutions of the bacteria into a freshly euthanised mouse. This is far from ideal; not only is it a waste of animals, but it is often difficult to compare between experiments as the bacteria are usually injected at slightly different depths. As an alternative, we have developed an assay in which labelled bacteria are contained within the wells of a microtitre plate which is then overlaid with slices of cooked ham to represent tissue. In this way we can vary the depth of the tissue by changing the number of slices of ham and show how this affects the bioluminescent signal and hence how many bacteria we are likely to be able to detect. This simple and elegant solution also makes financial sense, costing just a fraction of the traditional assay.

While still involving animals, we have also worked hard to explore the use of other organisms to minimise the use of mice in our studies. We now routinely use caterpillars of the wax moth *Galleria mellonella* which are easy to source and handle, and possess an innate immune response that protects them from most infections. The caterpillars are an ideal model host organism in studies which do not need the adaptive arm of the immune response to be present (Loh et al. 2013). For example, we now routinely use caterpillars as a first screen to determine if our bioluminescent bacteria are disabled in any way before infecting mice. We also use caterpillars to compare the virulence of different clinical bacterial isolates (Williamson et al. 2014), a task that could become prohibitive if it were to be done entirely using mice. Finally, we also use caterpillars as initial screens to test new potential antibiotics (Williamson et al. 2014).

Speaking up for animal research

One of the most valuable lessons I have learned is that scientists shouldn't feel they must keep their research from the public, just because it involves the use of animals. This gives the impression that we have something to hide. When the NC3Rs staff asked me to go public with my 3Rs award, I was initially worried I would become a target for anti-vivisectionists. With some gentle persuasion I agreed to have my name appear in the press, and underwent an intensive day of media training to prepare me for any media interest. But there was no media onslaught, just a small piece in *The Times* newspaper, under the headline "Award for being nice to mice". The experience taught me that I was wrong about the perceived hysteria that had previously stopped me from talking about my research in public. From that day I have gone on to combine being an active researcher with a growing passion for communicating science to the public, including talking about the use of animals and the Three Rs. I am a blogger, podcaster and radio commentator, and in 2011 worked with a professional graphic artist, Luke Harris, who animated a short script I had written about fireflies and my TB research. Uploaded to YouTube, "Meet the Lampyridae" (Wiles & Harris 2011) has had over 6,000 views to date. Another version of the animation (Wiles & Harris 2012) was also produced for the UK charity Understanding Animal Research for distribution to schools. Most rewarding though is the feedback I get from the public to my research and our commitment to the Three Rs. Recently, an audience member approached me after I had given a public talk and told me I had completely changed her opinion of the use of animals in research. She had never heard of the Three Rs and told me I had completely shattered all the myths she held about animal research. High praise indeed!

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