Research Design:
How to review as part of the protocol?

Dr Navneet Dhand

Senior Lecturer in Vet Biostatistics and Epidemiology

Faculty of Vet Science, The University of Sydney

navneet.dhand@sydney.edu.au

Scope of the talk: 3 Rs

The Three Rs (3Rs)

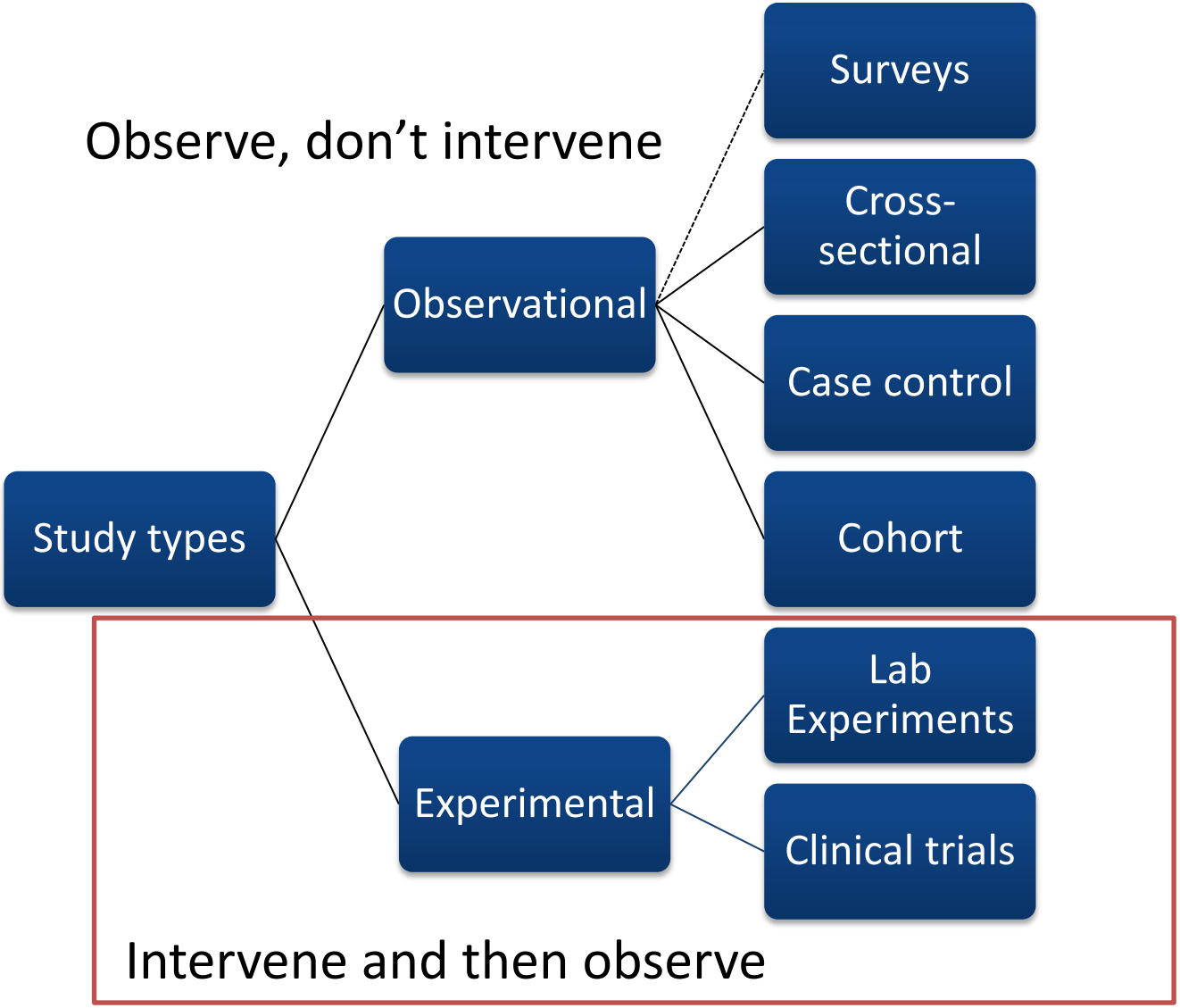
Replacement: methods or strategies that *replace or avoid* the use of animals in research and testing.

Reduction: reducing the numbers of animals used to the *minimum necessary* to achieve the scientific objectives, for example by improving experimental design and statistical analyses.

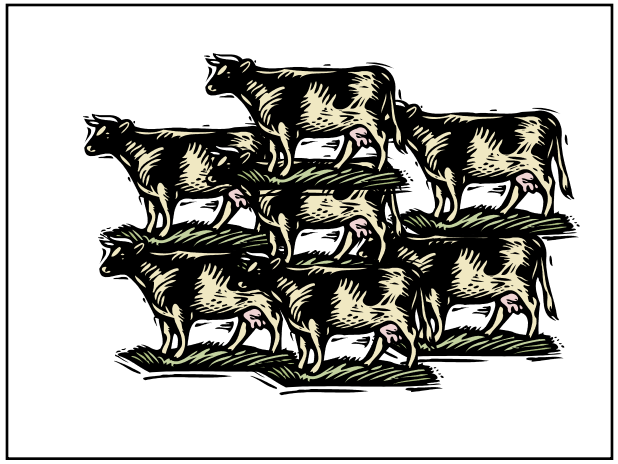
Refinement: refining scientific procedures and other factors affecting animals (for example, transport, housing, restraint) to *reduce suffering and improve the animals' welfare* at every stage of their lives.

Source: A resource book for lay members of ethical review and similar bodies worldwide

Scope of the talk: Study Designs



Animal Experiments



Apply
"Intervention"

Observe
the effect

The "intervention" can be anything that the investigators want to test

A drug, A vaccine, Temperature, Humidity, Feed,…

Example -I

- A pharmaceutical company wanted to test a new product for the treatment of osteoarthritis in dogs

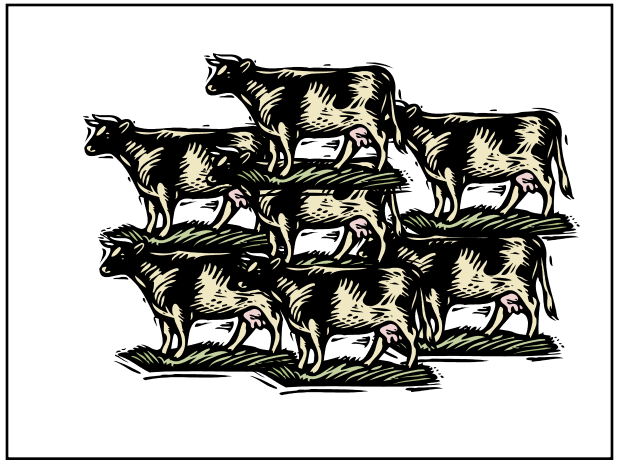
The new drug for arthritis is the "intervention"

Example - II

- Insufficient energy supply is a major cause of lamb mortality
- Lamboost® is a commercially available energy rich supplement
- Amanda wanted to know whether Lamboost® could help improve survival rates in lambs

Feed supplement Lamboost® is the "intervention"

Animal Experiments



Apply
"Intervention"

Observe
the effect

The "intervention" can be anything that the investigators want to test

A drug, A vaccine, Temperature, Humidity, Feed,…

How would you know that the intervention works?

Give “Lamboost”

How would you know that Lamboost improved survival?

Survival could be better just due to good season

New treatment for osteoarthritis

How would you know that the new is effective in reducing pain?

Condition of the dog could improve due to natural healing

http://slaughteringthesheep.files.wordpress.com/2009/12/lambs.jpg

Comparative/Control group is crucial!

Give Lamboost

Treatment Group

Calculate survival %

Compare survival between treatment and control groups

Don't give Lamboost

Control Group

Calculate survival %

Ethical issues in selection of a control group

- Is it ethical not to treat control animals?
  - Evaluation of a new treatment for arthritis
    - Some dogs treated with the new therapy
    - Some dogs not treated at all???? Is this ethical?
  - Lamboost trial? No problem for selecting controls
- Solution: Compare the new with a standard treatment
    - One group of dogs treated with the new therapy
    - The other group treated with the usual therapy → no ethical issues.

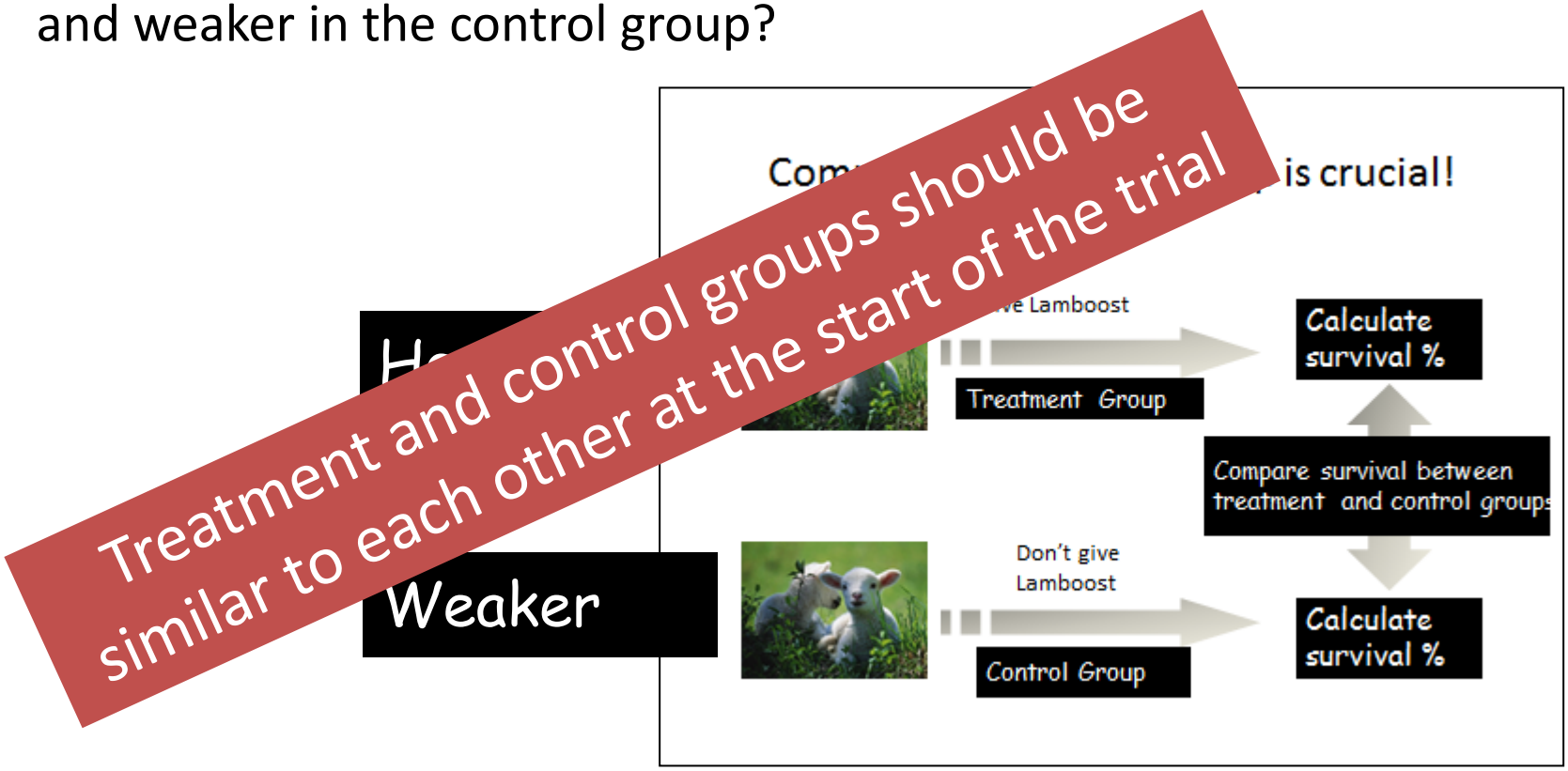
Check # 1

Did the investors propose a comparative/control group?

Would they select this group in an ethical manner?

Assume that the investigators did propose to select a control group…

- What would happen if healthier lambs are in the treatment group and weaker in the control group?



Treatment group would have greater survival just because they are healthier at the start, not due to Lamboost

How can we make treatment and control groups similar?

- Randomisation is the best approach
  - Assign animals to treatment and control groups, so that each animal has the same (or known) chance of being selected in the treatment group

- How to randomly assign animals to groups
  - Tossing a coin
  - Using random number tables
  - Using random numbers generated by a computer

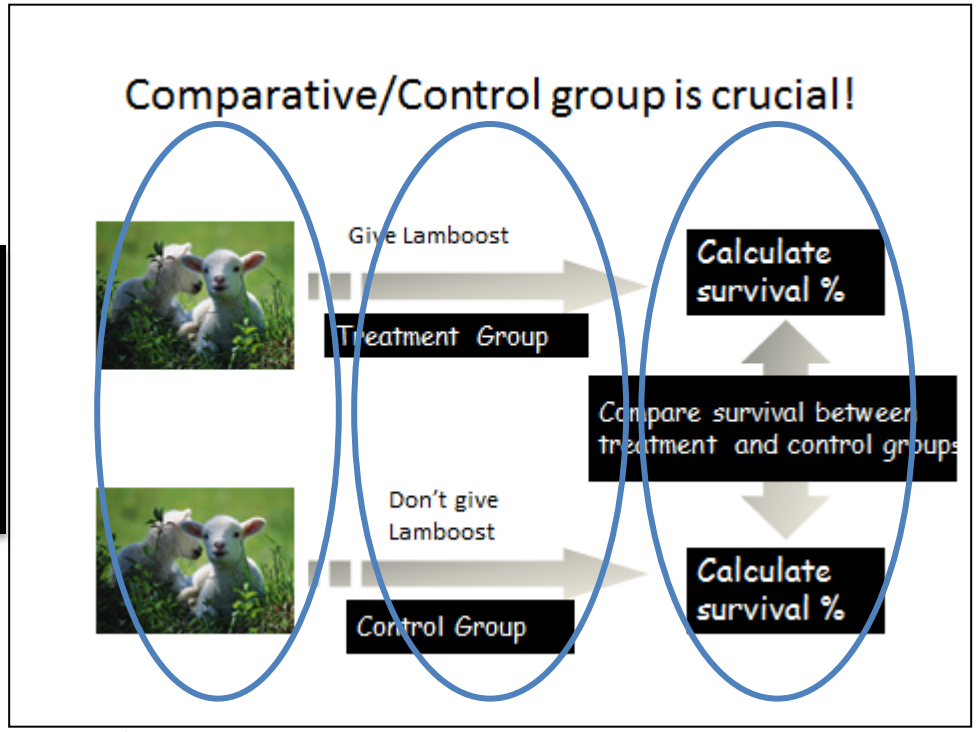
Check # 2

Did the investigators propose to randomly allocate animals into groups?

Assume that the investigators…

- √ selected a control group, and
- √ randomly allocated lambs to treatment and control groups

Is there still some possibility of obtaining biased results?



They removed bias in allocation

but there still could be bias in follow up and assessment

1. Bias in follow up

- When the controls are not cared for in a similar manner to the treated lambs, for example:
  - Treated lambs are given better feed than the controls
  - Controls are not dewormed while treated lambs are
  - Controls are not vaccinated but treated lambs are

- How to avoid this?
  - Blinding: conceal identification of animals from the research manager/farmer
  - Avoid coloured tags: e.g. green tags for treatment group and yellow for the control group

2. Bias in assessment of the outcome

- When the assessor knows who is in the treatment and the control group
  - Solution: Double Blinding – conceal identification of the animals from the assessor

- When outcome is a subjective measure: The assessor could record better results for the treated group
  - Solution: Use an objective method to measure the outcome e.g. mortality, weight gain, blood glucose
  - Really important if blinding is not possible

http://asserttrue.blogspot.com.au

Check # 3

Are there protocols in place for blinding of the research manager, if of course, it is feasible?

Are the proposed outcomes objective?
If not, are they going to practice double blinding?

Triple blinding

- Blind the statistician to the treatment → Triple blinded trials

Make sure that someone knows who is in the treatment and the control group!

Summary so far…

To reduce bias and improve animal welfare :

- Select a control/comparative group
- Randomly allocate animals into treatment groups
- Conceal identification of animals from the owner/research manager
- Use an objective test for assessment of the outcome
- Conceal identification of animals from the assessor (particularly important if a subjective test is used for assessment)

Experiment Results

- Survival rate in the treatment group is 7% higher than in the control group

- How would you know that this difference is real and not just due to random variation?
  - Conduct a statistical test
    - If the test is significant ➔ Conclude that the treatment is effective
    - If the test is non-significant ➔ Differences are due to chance

or

The investigators didn't select sufficient sample size to detect the differences!

Sample size: why does it matter?

- Too big?
  - Unnecessarily expensive → Wastage of resources
  - Unethical to use more animals than required

- Too small?
  - Experiment may not be able to achieve the power
    - Wastage of resources
    - Unnecessary pain and suffering for animals → Unethical

Research studies may have different objectives

- To estimate a proportion: e.g. a survey to estimate prevalence of a disease in an animal population
- To demonstrate disease freedom or detect a disease: e.g. to demonstrate that a farm is free from Q fever
- To compare proportions: e.g. a trial to compare pregnancy rates or mortality between groups (Paired or independent)
- To compare means: e.g. an experiment to compare liver copper levels or blood selenium levels between groups (Paired or independent)
- To compare time to an event: e.g. compare survival or recovery time after surgery

Different sample size formulae/calculators for all these objectives

http://statulator.com/

Sample size for comparing means

$$sample\ size \approx \frac{16\sigma^2}{d^2}$$

- Smaller the size of the difference the investigator wishes to detect, greater the sample size

Difference	Standard deviation	Sample size
10	20	64
5	20	256

- Greater the variation within groups, greater the sample size

Difference	Standard deviation	Sample size
10	20	64
10	40	256

Check # 4

Did they calculate sample size?
If yes, did they describe their assumptions and input values?

Summary: what to look for in a protocol?

- Did the investigators calculate sample size?
- Did they include a comparative or a control group?
- Did they propose to randomly allocate animals to treatment and control groups?
- Did they propose to blind the research manager of the treatment status of animals?
- Is their outcome objective? If not, would they blind the assessor of the treatment status of animals?

Guidelines

Preventive Veterinary Medicine 93 (2010) 11–18

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

The REFLECT statement: Methods and processes of creating Reporting Guidelines For Randomized Controlled Trials for livestock and food safety☆

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

The ARRIVE Guidelines

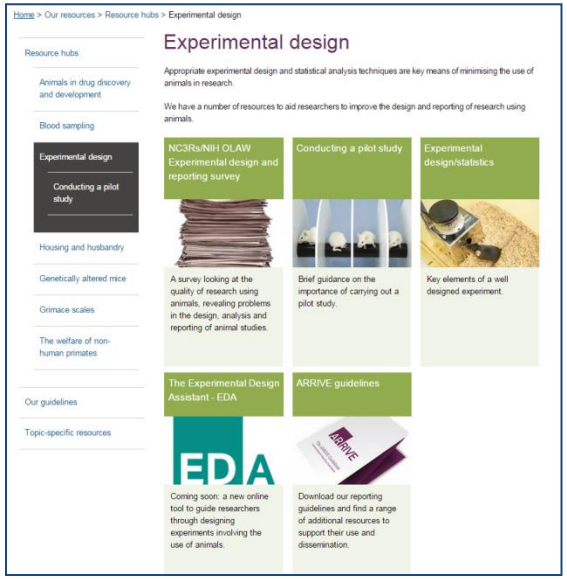
Animal Research: Reporting of *In Vivo* Experiments

Carol Kilkenny[1], William J Browne[2], Innes C Cuthill[3], Michael Emerson[4] and Douglas G Altman[5]

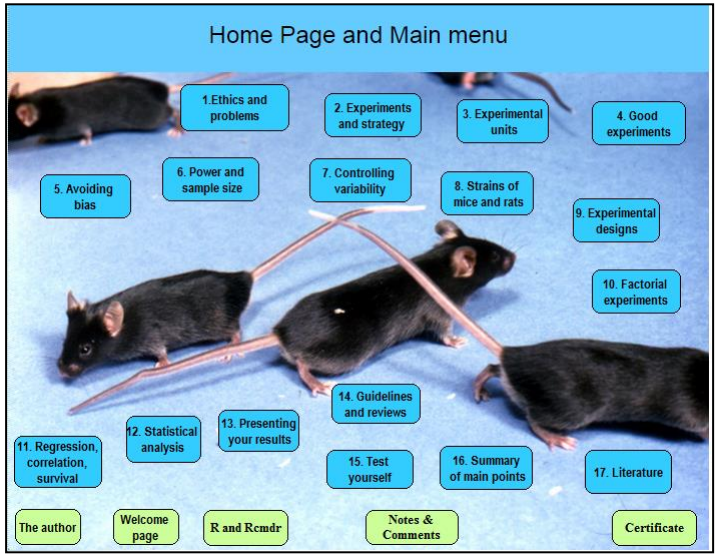
[1]The National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, UK., [2]School of Veterinary Science, University of Bristol, Bristol, UK, [3]School of Biological Sciences, University of Bristol, Bristol, UK, [4]National Heart and Lung Institute, Imperial College London, UK, [5]Centre for Statistics in Medicine, University of Oxford, Oxford, UK

The ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines were developed as part of an NC3Rs initiative to improve the design, analysis and reporting of research using animals – maximising information published and minimising unnecessary studies. The guidelines were published in the online journal *PLOS Biology* in June 2010 and are currently endorsed by scientific journals, major funding bodies and learned societies.

Resources

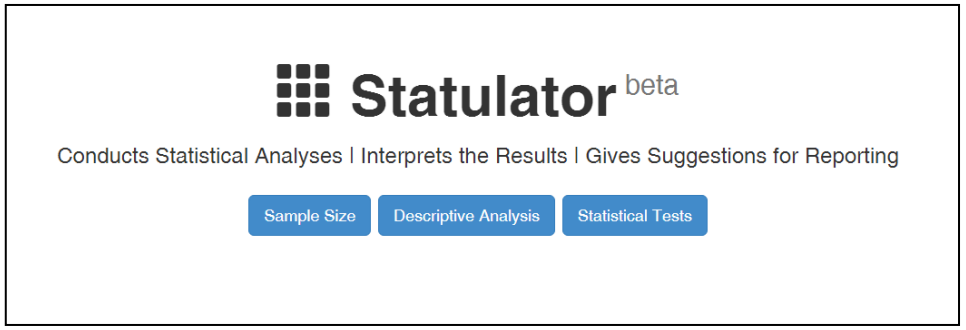


http://www.nc3rs.org.uk/experimental-design



http://www.3rs-reduction.co.uk/html/main_menu.html

http://www.felasa.eu/recommendations/reports/principles-and-practice-in-ethical-review-of-animal-experiments-across-euro/



http://statulator.com/