Harmonisation of the Care and Use of Agricultural Animals in Research

Application of the 3Rs to challenge tests used in vaccine development and quality control

(with a focus on Humane Endpoints)

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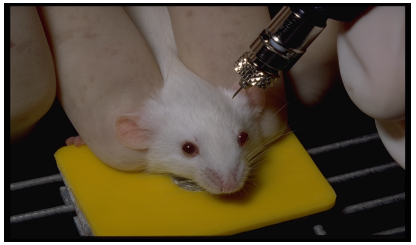
Agenda

- What are challenge tests?
- Historical context
- Vaccine production and quality control
- Three R opportunities
- Humane endpoints
  - How to assess humane endpoints
  - Organisational aspects of humane endpoints
  - Dilemma's

Scope presentation

Challenge : to inject an experimental animal (immunized with a test substance) with disease micro-organisms to test for immunity to the disease



Focus is on vaccines, but with references to all experiments where animals are experimentally infected with a disease micro-organism

- in vaccine development and in vaccine batch release testing (safety, efficacy)
- in development of anti-inflammatory drugs and antibiotics
- to study specific infectious diseases (pathogenesis, clinical course, prevention, treatment)
- diagnostic testing
- a.o.

Challenge tests in the historical context

Poilly-le-Fort, France, 1881: Louis Pasteur: first reported challenge experiment. Five weeks after vaccination of 24 sheep and one goat with a live attenuated Anthrax vaccine, all animals and 29 control animals were challenged with a viruent strain of anthrax. All immunised animals survived while all non-vaccinated animals died.

Infection models in the historical context

Koch's Postulates (1884) : describe criteria to establish causal relationship between disease and micro-organism believed to be responsible

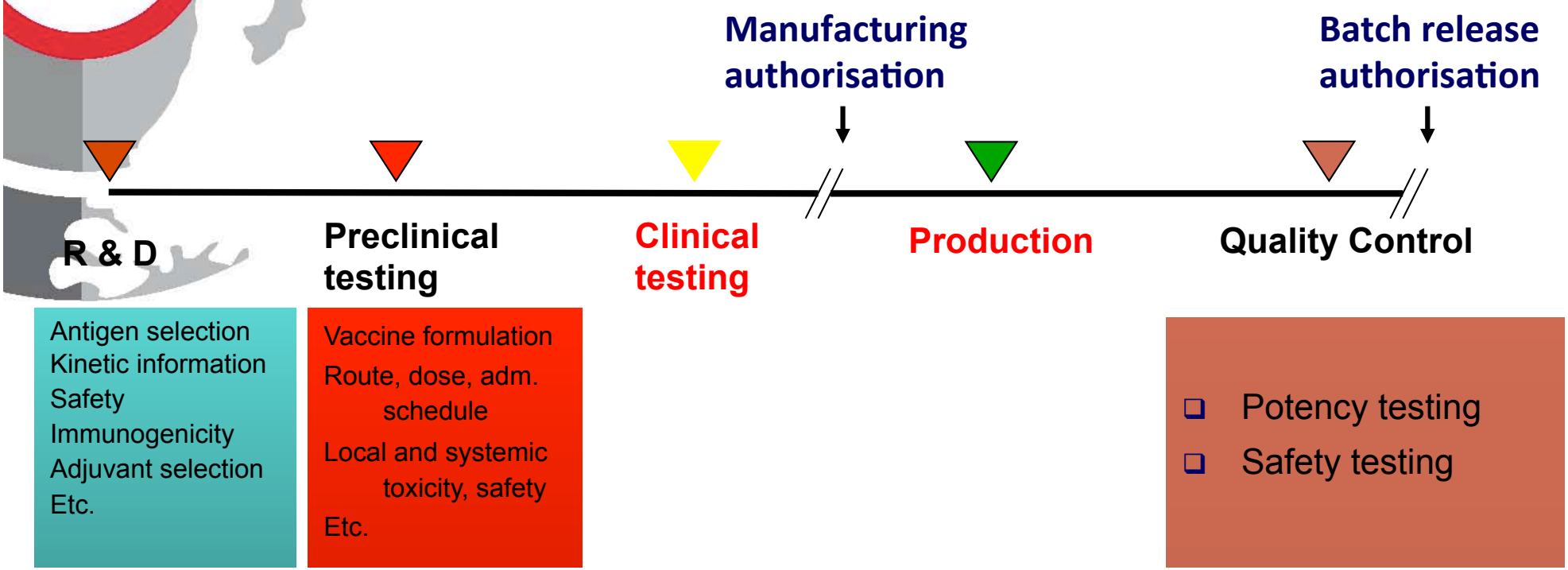
- Isolation of possible causal micro-organism from diseased person
- Pure culture of micro-organism on culture plate
- Injection of resulting pure culture into healthy and **susceptible experimental animals** : characteristic clinical signs in these animals
- Reisolation of the diseased host must be identical to the original micro-organism

Facts & Figures

	Animal use	Vaccines	Infections
Total	**575,278**	**96,678**	**66,037**
Horse	**2,976**	**2,376**	**138**
Pig	**10,516**	**3,645**	**1,353**
Sheep	**3,021**	**213**	**50**
Cow	**4,598**	**508**	**325**
Chicken	**81,704**	**35,325**	**17,747**
Fish	**29,417**	**60**	**700**
Mouse	285,215	36,264	39,733
Rat	120,296	5,541	3,001
Guinea pig	4,857	4,105	84
Hamster	3,963	2,542	2,062

Figures from the Netherlands, 2010

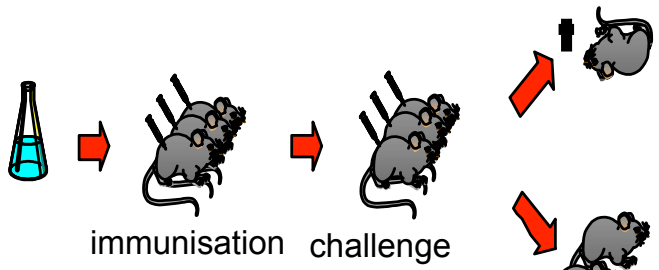
Stages in a vaccine life and animal use



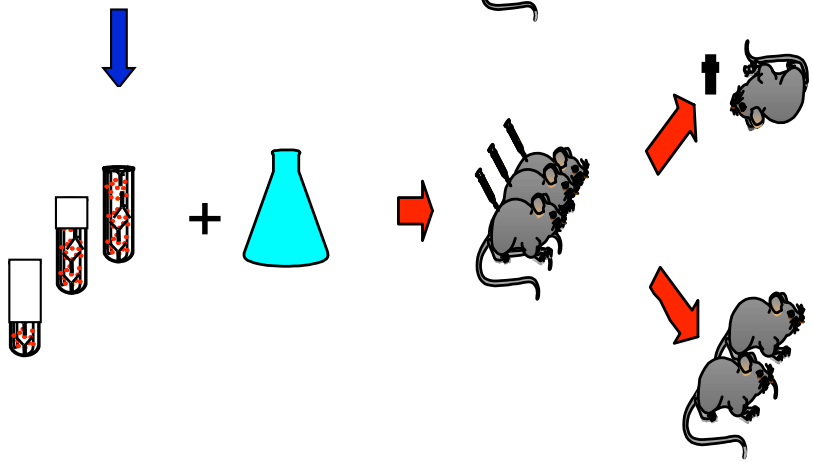
Comments

- Animals are used in vaccine R&D, in preclinical testing and in batch quality control
- R&D and preclinical testing is performed once only, batch control routinely
- If possible, surrogate models (e.g. rodents) are used in all phases, apart for chicken and fish vaccines. Large farm animal use is restricted to (R&D), preclinical testing and safety testing

The challenge procedure in vaccine potency testing



Direct challenge procedure



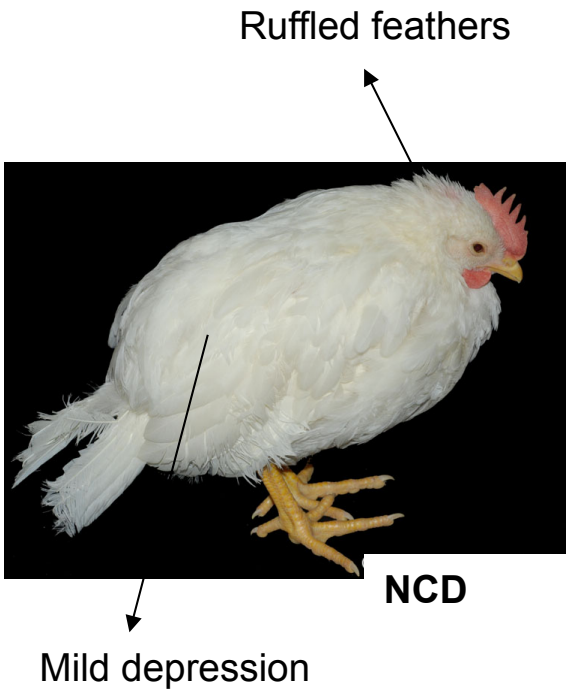
Indirect challenge procedure

Characteristics challenge test

- Extensive animals use
- Severe pain and suffering for significant part of animals
- Statutory required

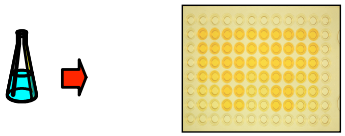
Reasons to promote Three R alternatives

- Animal welfare concern: high pain & distress levels
- Legal: ‘Animals have an intrinsic value…and should be treated as sentient creatures.’ (Directive 2010/63/EU, recital 12)
- Time cosuming and expensive
- Safety concern: virulent micro-organisms in the animal lab.



Replacement and challenge procedures

Alternative approaches

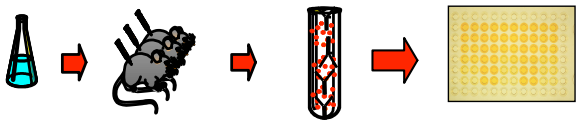


Replacement

- Antigenicity testing
  - NewCastle Disease (NCD) vaccine
- Consistency testing

Reduction and challenge procedures

Alternative approaches



Replacement

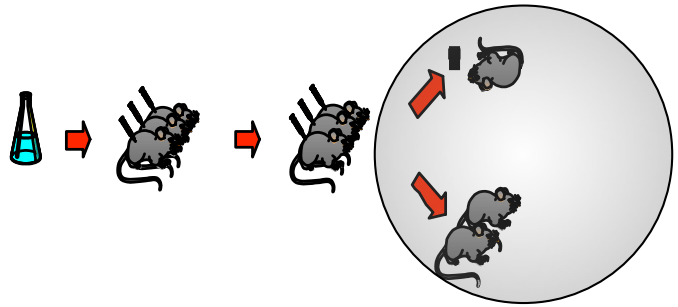
- Antigenicity testing
  - NewCastle Disease (NCD) vaccine
- Consistency testing

Reduction

- Serological testing (no challenge)
  - Erysipelas vaccine
- Freeze drying challenge culture
- Reduction of dose groups
- Retesting policies
- etc.

Refinement and challenge procedures

Alternative approaches



Replacement

- Antigenicity testing
- Consistency testing

Reduction and Refinement

- Serological testing (no challenge)
- Freeze drying challenge culture
- Reduction of dose groups

Refinement

- Optimising housing conditions
- Providing pain relief??
- Humane endpoints

Humane endpoints: legal driver

Council Directive 2010/63/EU

Art.4.3: Member States shall ensure refinement of breeding, accommodation and care, and of methods used in procedures, **eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm** to the animals

Art.13.3: Death as an end-point of a procedure shall be avoided as far as possible and replaced by **early** and **humane end-points**.

Where death as the end-point is unavoidable, the procedure shall be designed so as to:

a) result in the death of as few animals as possible; and

b) reduce the duration an intensity of suffering to the animal to the minimum possible.

Humane endpoints

‘The earliest indicator in an animal experiment of (potential) pain and/or distress that, within its scientific context and moral acceptability can be used to avoid or limit such consequences by taking actions such as humane killing or terminating or alleviating the pain and distress’

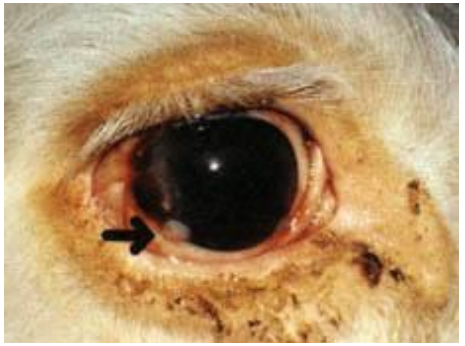
(Cost Manual of Laboratory Animal and Use, 2010)

Balancing scientific needs vs humane endpoints

- Earlier, more humane endpoints should not distort experimental outcomes, thereby invalidating the experiment.On the other hand, however, pain and distress might be a confounding factor.

- Shifts to earlier endpoints can be validated by correlation with experimental biomarkers

- Seeking earlier endponts must be a dynamic process, involving all those having a responsibility in the experiment (PI, research staff, ethics committee, attending veterinarian, animal welfare body, technicians)

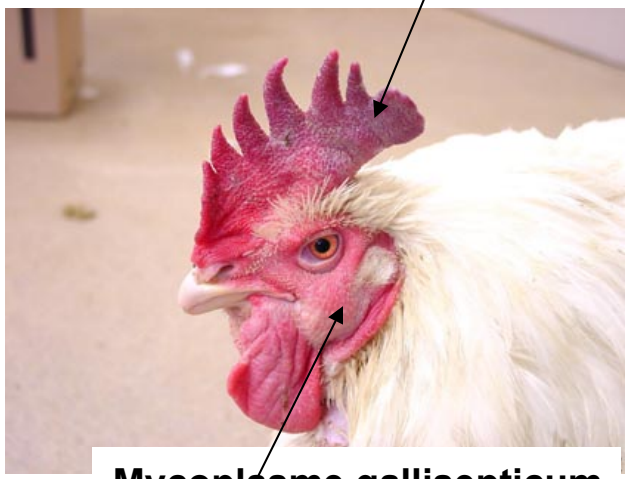


Ocular cancer cattle: skin carcinoma

NCD

Types of parameters for humane endpoints

- Clinical signs :
  depending on type of experiment; e.g. neurological signs, poor condition, paralysis, dehydration, cyanosis, etc.
- Pathophysiological parameters :
  body temperature, body weight, respiratory rate, etc.
- Behavior parameters :
  stereotypic behavior, agression, depression, etc.
- Hormone levels :
  prolactine, corticosteroids.
- Haematological and micro-biol. parameters :
  Hb, Hematocryte, IgG, leucocytes, virus titres, etc.



Mycoplasme gallisepticum

How to assess humane endpoints

- **Observation and monitoring**
  - clinical signs, respiration, neurological signs, etc.

- **Biomarkers**
  - blood, urine, saliva, tissue, tears, hair

- **Microchip implant systems & Non-invasive technologies**
  - Telemetry (Temp., heart rate, blood pressure, etc.)
  - Biophotonic imaging

Observation and monitoring

Vital assessments in observaton

- ✓ Appearance
- ✓ Posture
- ✓ Spontaneous behavior
- ✓ Provoked behavior
- ✓ Clinical signs
- ✓ Body weight

When observing

Don’t just look but observe!

- ➢ Start looking at the group (undisturbed behavior)
- ➢ Include the environment
- ➢ Know your animals and signals of good health
- ➢ Look and compare
- ➢ Be openminded

Use of a score sheet

Animal number **Time:**	**Day 1**	**Day 1**	**Day 2**	**Day 2**	**Day 3**	**Day 3**
		X to XXX: from slight to severe				
<u>Undisturbed observation:</u>						
Condition		x	x	xx	xxx	†
Social interaction			x	x	xxx	
Ruffled feathers		x	xx	xx	xxx	
Neurological signs			X	X	XX	
Posture & mobility			x	xx	xx	
<u>Response on handling</u>						
Vocalisation				xx		
Agression			x	xx	xxx	
<u>General clinical signs</u>						
Body weight			x	xx	xxx	
Faeces/urine		x	xxx	xxx	xxx	
Etc.						

Alert criteria : criteria that should trigger frequent observation
Decision criteria : criteria that should be used to apply humane endpoints

How to assess humane endpoints

- **Observation and monitoring**
  - clinical signs, respiration, neurological signs, etc.

- **Biomarkers**
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- **Microchip implant systems & Non-invasive technologies**
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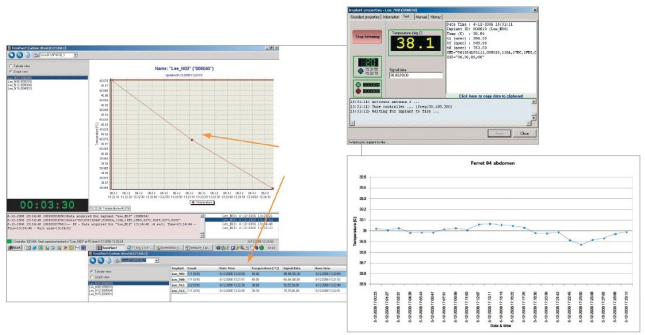
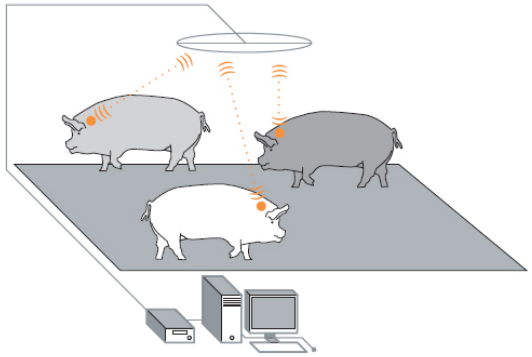
Microchip implant systems & Non-invasive technologies

Possible parameters for humane endpoints

- ✓ Temperature
- ✓ Activity
- ✓ Pressures (blood, ventricular, ocular, pleural,
- ✓ intra-cranial, etc.)
- ✓ ECG (heart rate, QT interval)
- ✓ EMG
- ✓ EEG
- ✓ Blood flow



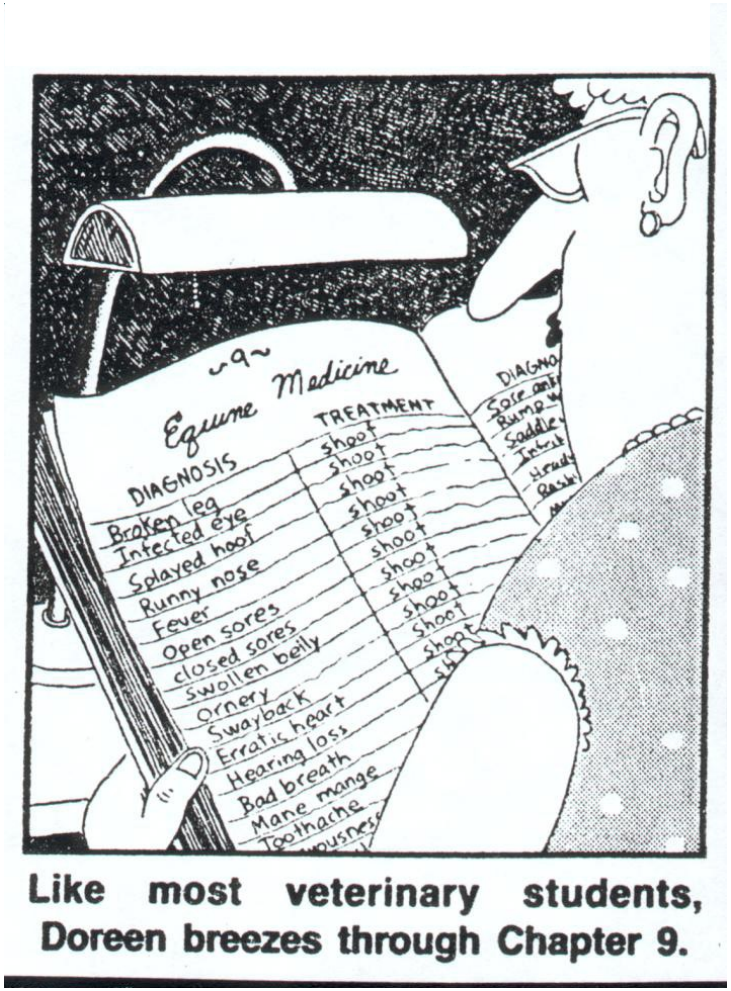
Pictures provided by TeleMetronics Biomedical



A basic TemPlant system contains a number of implants,a receiver, an antenna, and TempControl software on a PC.

DEFINITION: The earliest indicator in an animal experiment of (potential) pain and/or distress that, within its scientific context and moral acceptability can be used to avoid or limit such consequences by taking actions such as humane killing or terminating or alleviating the pain and distress'

- Euthanise the animal when relevant humane endpoints are reached
- Euthanise animal in case pain & ditress is not related to the experimental procedure
- Sedate the animal(s)
- Apply analgesics
- Stop the experimental procedures



Organisational aspects of applying humane endpoints

Before the experiment

- Identify expected clinical progress, vital clinical signs and critical steps/time points (literature review/pilot study!)
- Identify type of parameter(s) to be used and what frequency of observations will be
- Discuss these with team (animal technicians, animal welfare off., pathologist)
- Define responsibilities of staff
- If needed, train staff

During the experiment

- Use score sheets
- Observe animals daily (more frequent when needed) and up-date score sheets
- Communicate on findings

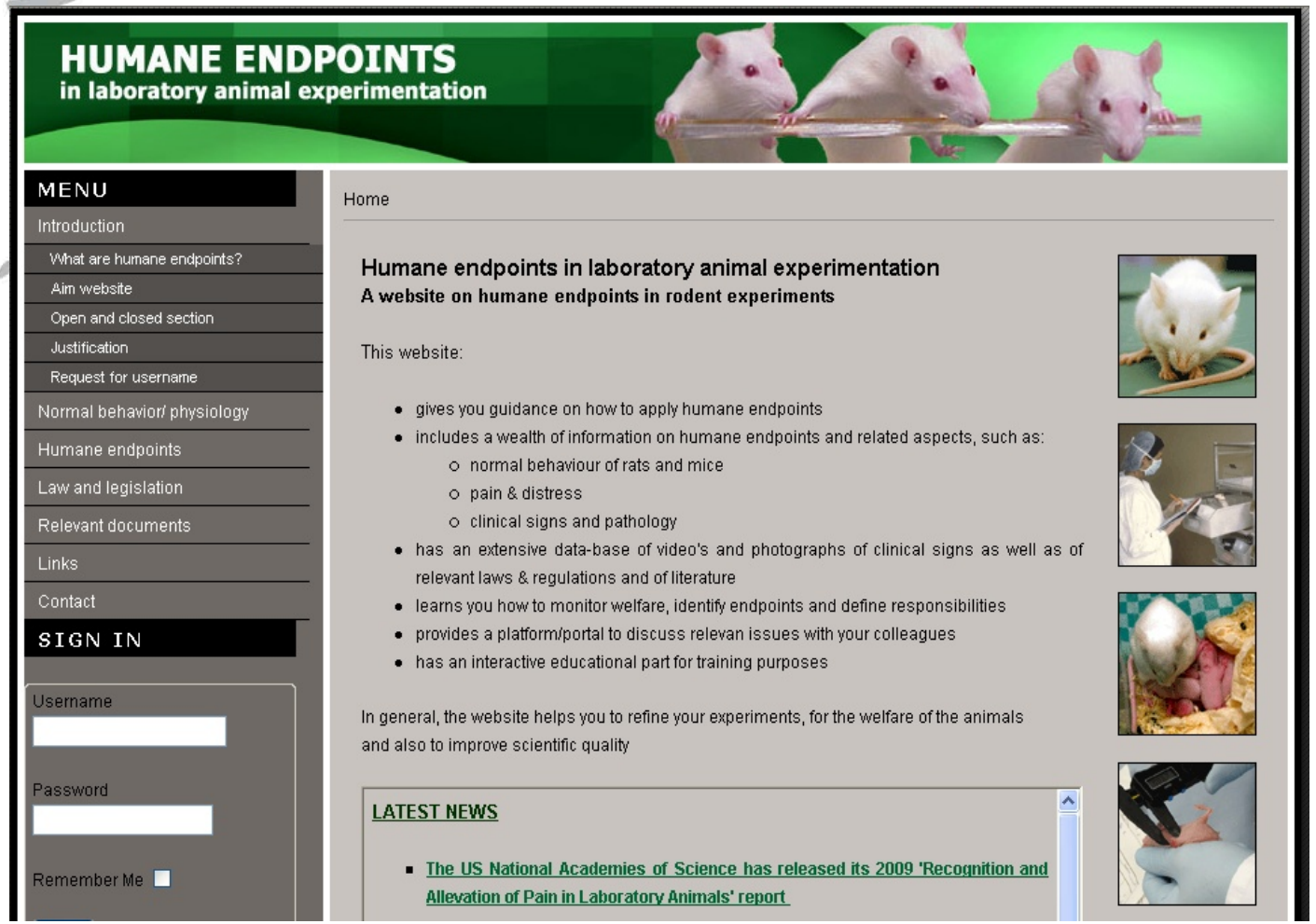
After the experiment

- Include pathologist in evaluation
- If possible, modify parameters and criteria
- Publish information

Dilemma's in applying humane endpoints

- Observing the animals for 24 hrs/day?
- Dealing with investigators who want to have the maximum of information?
- Handling diseased animals and providing adequate housing?
- Sedate animals or provide analgesics to animals in pain?
- Killing or treating an animal?

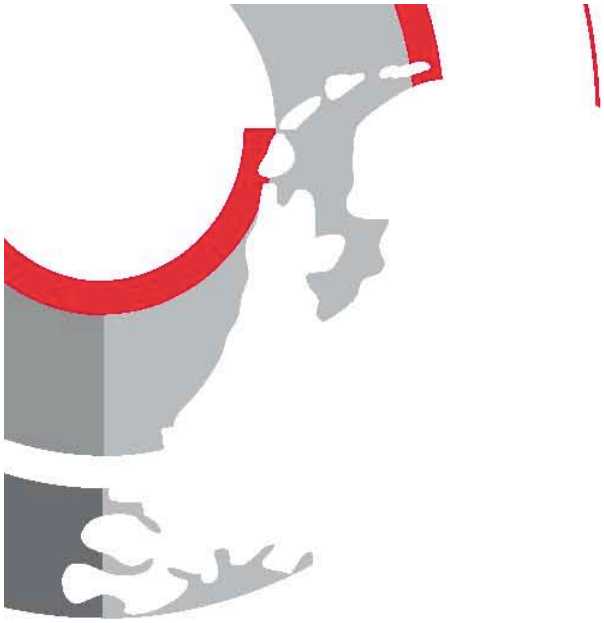
Website Humane Endpoints



www.humane-endpoints.info

Take home message

- Applying 3Rs in challenge models is not an option but a must
- Consider first Replacement. Reduction and refinement are second best
- Humane endpoints are the last resort of a 3R's approach
- Be aware of training needs
- Clearly define responsibilities
- Be a driver. Make use of new technologies



Thanks for your attention!