

2nd International Conference on Humane Endpoints in Animal Experiments for Biomedical Research

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Bundesinstitut für Risikobewertung
Federal Institute for Risk Assessment
Berlin, Germany

Animals in Infectious Disease Research: Balancing Welfare, Humane Endpoints and Valid Scientific Outcomes

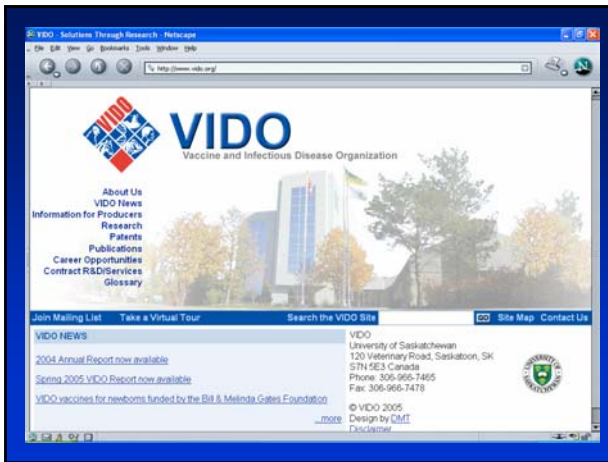


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“... deciding which is the most suitable course to adopt when it is found necessary to ‘smooth the path to death’.”

Dr E Wallis Hoare, *A System of Veterinary Medicine* 1914

Humane Endpoints in Infectious Disease Research



Animal Use in Infectious Disease Research – Historical



Koch's Postulates, 1882

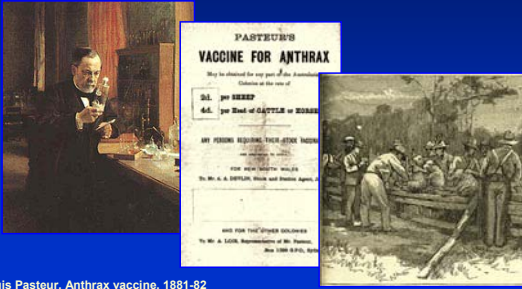
To prove an organism was the cause of any disease it was necessary to demonstrate:

- That the organism could be discoverable in every instance of the disease;
- That, extracted from the body, the germ could be produced in a pure culture, maintainable over several microbial generations.
- That the disease could be reproduced in experimental animals through a pure culture removed by numerous generations from the organisms initially isolated;
- That the organism could be retrieved from the inoculated animal and cultured anew.

Robert Koch, Tuberculosis, 1882



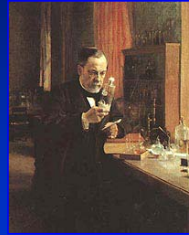
Animal Use in Infectious Disease Research – Historical



Louis Pasteur, Anthrax vaccine, 1881-82



Animal Use in Infectious Disease Research – Historical



Dr Roux said of Pasteur:

"...Pasteur, who had been obliged to sacrifice so many animals in the course of his beneficent studies, felt a veritable repugnance toward vivisection...."

"...if the animal cried a little, Pasteur immediately felt pity and lavished on the victim consolation and encouragement...."

Louis Pasteur, Anthrax vaccine, 1881-82

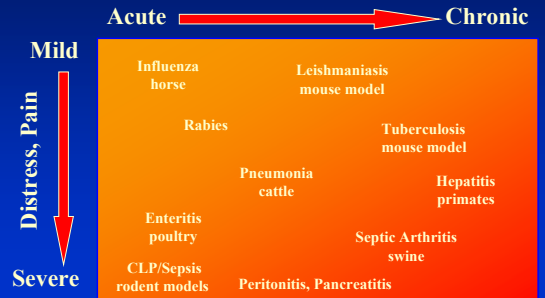


Animal Use in Infectious Disease Research – Today

- studying the immune system
- studying genetic control of immune system factors
- studying genetic control, functions, and interactions of the many immune cell types
- studying the process of inflammation
- studying specific infectious diseases
 - pathogenesis; clinical course; prevention; treatment
- development of vaccines and immune modulators
- efficacy testing; safety testing; virulence testing
- diagnostics



Animal Use in Infectious Disease Research - Models



Animal Models Used in Infectious Disease Research

- Peracute Infectious Disease
 - Cecal Ligation & Puncture (CLP) Model, rodents
 - model of sepsis
- Acute Infectious Disease
 - Acute Viral/Bacterial Respiratory Disease in Cattle
- Chronic Infectious Disease
 - Cutaneous Leishmaniasis in Mice



Balancing Scientific Need for Objective Outcomes with Ethical Responsibility to Minimize Distress, Pain and Suffering

- Earlier, more humane endpoints should not distort experimental outcomes, thereby invalidating the experiment(s)
- Shifts to earlier endpoints can be validated by correlation with experimental biomarkers
- Seeking earlier endpoints must be a dynamic process, involving the principal investigator and research staff, the animal ethics/care committee, and the attending veterinary staff



- CCAC POLICY STATEMENT - ETHICS OF ANIMAL INVESTIGATION (1989)

“Animals must not be subjected to unnecessary pain or distress. The experimental design must offer them every practicable safeguard...”

“If pain or distress is a necessary concomitant to the study, it must be minimized both in intensity and duration...”



- GENERAL ENDPOINTS GUIDELINE

In experiments involving animals, any actual or potential pain, distress, or discomfort should be minimized or alleviated by choosing the earliest endpoint that is compatible with the scientific objectives of the research. Selection of this endpoint by the investigator should involve consultation with the laboratory animal veterinarian and the animal care committee.



Process for Establishing Humane Endpoints

A. The animal use protocol form and protocol review

- Make sure the animal use protocol form section on endpoints asks the right questions about:
 - the expected clinical signs of the progressing condition, and;
 - the monitoring and checklist developed for this condition.

CCAC Endpoints Guidelines include a list of suggested questions which can be asked on the protocol form or during protocol review.



- What are the scientific justifications for using the proposed endpoint?
- What is the expected time course for the animals, from initial treatment to first signs of pain/distress, to the death of the animal, based on previous information with the specific model under study?
- When are the effects to the animal expected to be the most severe?
- If the course of the disease and expected signs of the adverse effects are unknown, could an initial (pilot) study, under close observation by the investigator and/or laboratory animal veterinary staff, answer these questions?
- Has a checklist of observations, on which the endpoint will be based, been established?



- Who will monitor the animals (identify all responsible) and keep records?
- Has a clear chain for reporting observations been established?
- What will be the frequency of animal observations: a) during the course of the study; and b) during critical times for the animals?
- Do the investigators, animal care and technical staff have the training and expertise to monitor the animals adequately?
- What provisions have been made to deal with any animals that show unexpectedly severe signs and symptoms?
- For toxicological studies, have existing toxicological data been evaluated?



Process for Establishing Humane Endpoints

A. The animal use form and protocol review process

- Any observation checklists provided by the principal investigator should be carefully reviewed for:
 - the actual observations to be made
 - the frequency of monitoring
 - the stated “endpoint” based on presence of, or scoring of, the observations



Process for Establishing Humane Endpoints

- B. For new infectious disease models, or when the research team has little or no experience
- consider approving only a **pilot study** to set observations, monitoring times and endpoints



Process for Establishing Humane Endpoints Pilot Studies

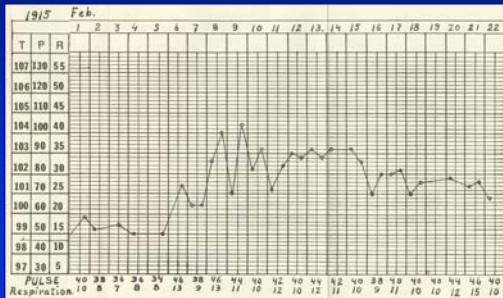


FIG. 106. HORSE SUFFERING WITH SWAMP FEVER (after Udall and Fitch).

Udall and Fitch. 1915. Preliminary report on the recognition of swamp fever or infectious anemia in New York State. Cornell Veterinarian, Vol V, p 69.



Process for Establishing Humane Endpoints Pilot Studies



Process for Establishing Humane Endpoints

- C. Gathering information about a new infectious disease model

- Understanding the science and model, critical for the animal ethics committee and laboratory animal veterinarian

- literature searches
- scientific journal articles
- web searches

• **Monitoring schedules and endpoints rarely published**



Process for Establishing Humane Endpoints

- C. Gathering information about a new infectious disease model

Look at **same condition in humans**

including **treatments used in humans**

Symptoms Related to Septic Shock

1. Early: fever, anxiety, pain
2. Fever, tachypnea, fluid shifts, pain
3. Later: hypothermia, hypoxemia, hypotension
4. Terminal: altered consciousness (coma)



Process for Establishing Humane Endpoints

- D. Determining Vital Observations for Infectious Disease Model Checklist



Why does the behaviour of an animal change when it is sick, in distress, or in pain?

- Some changes in physiology and behaviour related to the effects of an **infectious disease** (or cancer), are the result of internal biochemical changes during the **Acute Phase Response**.

Gregory NG. 1998. Physiological mechanisms causing sickness behaviour and suffering in diseased animals. *Animal Welfare* 7:293-305



Acute Phase Response and Sickness Behaviour

Infection Inflammation

Activates local WBC and Stromal Cells

Production and release of cytokines and other mediators

Physiological responses to infection, cancer, trauma



Acute Phase Response and Sickness Behaviour

Infection Inflammation

Activates local WBC and Stromal Cells

cytokines and other mediators

IL6 & TNF act in brain to produce

Physiological responses to infection, cancer, trauma

Fever
Inappetance (and weight loss)
Lethargy (sleepiness)
Hyperalgesia



Acute Phase Response and Sickness Behaviour

Infection Inflammation

Activates local WBC and Stromal Cells

Production and release of cytokines and other mediators

Physiological responses to infection, cancer, trauma

Fever
Inappetance (and weight loss)
Lethargy (sleepiness)
Hyperalgesia

Neutrophilia
WBC adhesion
↑ CD4+, CD8+, NK cells
↑ Antibody production



Process for Establishing Humane Endpoints

D. Determining Vital Observations for Infectious Disease Model Checklist

General Observations for All Infectious Disease Models

- Temperature change
- Weight loss
- Behaviour changes

Specific Clinical and Physiological Observations

- Related to that particular animal model and condition



Process for Establishing Humane Endpoints

D. Determining Vital Observations for Infectious Disease Model Checklist

Monitoring Temperature Change

- At least daily recording
- For rodent models
 - Rectal probes
 - Implanted microchips
 - Infrared thermometers
 - Surface probe
- For large animal models
 - Various methods





Process for Establishing Humane Endpoints

D. Determining Vital Observations for Infectious Disease Model Checklist

Monitoring Temperature Change in Rodent Models

Fever (hyperthermia) not observed in many infections, or is transient

Hypothermia of 4-6°C associated with death outcome in some bacterial and viral infections in mice

In endotoxin injection hypothermia associated with death is lower (1°C below normal)



Process for Establishing Humane Endpoints

D. Determining Vital Observations for Infectious Disease Model Checklist

Monitoring Weight Loss

- Daily recording
- Reflects inappetence or inability to eat and drink
- Rate, duration and extent of weight loss important in setting endpoint



Process for Establishing Humane Endpoints

D. Determining Vital Observations for Infectious Disease Model Checklist

Monitoring Behaviour Changes

- Daily monitoring
- Decreased activity: lethargy, depression

For rodent models

Monitor activity during the "night" part of daily cycle, when most active



Process for Establishing Humane Endpoints

D. Determining Vital Observations for Infectious Disease Model Checklist

Specific Clinical and Physiological Observations

- Related to that particular animal model and condition



Endpoints Set and Protocol Approved!



Monitoring Compliance

- Go look at the animals
- Look at the monitoring records - the checklists
- Compliance Officers?





Monitoring Compliance

Monitor **mortality**

Ensure reporting to clinical veterinarian

University Committee on Animal Care and Supply
 Morbidity/Mortality Incident Report Form: Lab Animal Services Unit
 For Urgent Matters Contact the Animal Resources Centre: 966-4125
 (After Hours Call Campus Security: 966-5555)

Date: _____ Primary Investigator: _____ Protocol #: _____
 Department: _____ Phone: _____ Animal Location/Room #: _____
 Animal: Species and Strain _____ Age _____ Sex _____ Other _____
 Experimental History (information from cage card, recent treatment, etc.): _____



Animal Models Used in Infectious Disease Research

- Peracute Infectious Disease
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 - model of sepsis
- Acute Infectious Disease
 - [Acute Viral/Bacterial Respiratory Disease in Cattle](#)
- Chronic Infectious Disease
 - [Cutaneous Leishmaniasis in Mice](#)



Endpoints in Peracute Infectious Disease – Humane?

- [Cecal Ligation & Puncture \(CLP\) Model, rodents](#)
model of sepsis
- General procedure: Anesthesia and abdominal surgery; cecum ligated, and needle puncture made through and through the cecal wall to allow “leakage” contamination of the peritoneal cavity
- General clinical course 36-48-72 hours, depending on size and number of punctures, +/- administration of antibiotics



Endpoints in Peracute Infectious Disease – Humane?

- [Cecal Ligation & Puncture \(CLP\) Model, rodents](#)
model of sepsis

Course: 0 hour ————— 36-48 hours



Endpoints in Peracute Infectious Disease – Humane?

- [Cecal Ligation & Puncture \(CLP\) Model, rodents](#)
model of sepsis
- [Frequency of monitoring?](#)
 - every 4-6 hours
- Dilemmas:
- What clinical observations can be used to accurately assess the animal's rapidly deteriorating condition?
- How to deal with related pain plus general, non-specific “sickness” and “suffering”, without affecting the scientific results?



Endpoints in Peracute Infectious Disease – Humane?

- [Cecal Ligation & Puncture \(CLP\) Model, rodents](#)
model of sepsis
- Possible Options to Minimize Pain and Distress:
 - 1a). Approve pilot studies using shortest time points first, proceeding from there
 - 1b). Ensure **research endpoint(s)** clearly defined, and immune cell types of specific research interest identified (e.g., recruitment of macrophages into lung)



Endpoints in **Peracute** Infectious Disease – Humane?

- Cecal Ligation & Puncture (CLP) Model, rodents
model of sepsis
- Possible Options to Minimize Pain and Distress:
 2. Keep animal **sedated / anaesthetized / provide analgesics for the duration**, using drugs that have least impact on specific research endpoint or immune cell type (may need separate pilot study or additional treatment group to assess impact of drugs)



Endpoints in **Peracute** Infectious Disease – Humane?

- Cecal Ligation & Puncture (CLP) Model, rodents
model of sepsis
- Possible Options to Minimize Pain and Distress:
 3. Alternate Model? Injection of Endotoxin
Is injection of endotoxin (LPS) to initiate immune responses modeling "septic shock" a more humane procedure than sepsis induced by surgical Cecal Ligation & Puncture?



Humane Endpoints in **Acute** Infectious Disease

- Acute Viral/Bacterial Respiratory Disease in Cattle
Model of viral-bacterial synergistic infection in farm conditions, associated with Bovine Respiratory Disease outbreaks in feedlot calves
- Beef calves 5-6 months of age, maintained in outdoor pens
- Calves infected with BHV-1; then infected with either *Mannheimia haemolytica* or *Haemophilus somnus* 4 days later (aerosol challenge)
- General clinical course: signs of respiratory infection appear 3-4 days after bacterial challenge; worsen in the next 2-3 days



Humane Endpoints in **Acute** Infectious Disease

- Acute Viral/Bacterial Respiratory Disease in Cattle



Humane Endpoints in **Acute** Infectious Disease

- Acute Viral/Bacterial Respiratory Disease in Cattle
- Clinical Evaluation and Observational Checklist
Monitoring schedule: Two documented observations **daily**
 - One complete clinical assessment, including temperature and body weight – veterinary
 - One observation – veterinary and/or animal care technician
- Clinical scoring of signs, detailed descriptors
 - Rhinitis or nasal score (0 = normal; 4 = very severe rhinitis)
 - Respiratory distress score (0 = normal; 4 = very severe distress)
 - Strength score (0 = normal; 4 = recumbent, unable to get up)
 - Depression score (0 = normal; 4 = moribund, comatose)



Humane Endpoints in **Acute** Infectious Disease

- Acute Viral/Bacterial Respiratory Disease in Cattle
- Overall Sickness Score assigned by clinical veterinarian
 - determined from daily clinical evaluation and observation scoring
0 = healthy animal; 4 = moribund
- Endpoint set at 3 in the overall sickness score
 - Euthanasia, and necropsy



Humane Endpoints in Chronic Infectious Disease

- **Cutaneous Leishmaniasis in Mice**

Model used to study immunotherapy (vaccination; immunomodulation) of chronic *Leishmania major* infection

Model: Mice infected with organisms in: a) the skin of the dorsal flank region; b) footpad

General clinical course: lesions develop 5-6 weeks following infection, expand in next few weeks



Humane Endpoints in Chronic Infectious Disease

- **Cutaneous Leishmaniasis in Mice**

Model used to study immunotherapy (vaccination; immunomodulation) of chronic *Leishmania major* infection

Documented monitoring schedule: Weekly for first four weeks, then daily

Agreed-upon endpoints:

Dorsal flank skin: when lesion reaches 1cm diameter

Footpad: when foot pad thickness increases 1.5mm



Humane Endpoints in Chronic Infectious Disease

- **Cutaneous Leishmaniasis in Mice**

Model used to study immunotherapy (vaccination; immunomodulation) of chronic *Leishmania major* infection



Minimizing the Impact of the Infectious Disease on the Experimental Animal's Welfare

- Ensuring the attitudes and skills of people
• Training programs essential!

Screenshot of the CCAC website showing the National Institutional Animal User Training Program (1999) page. The page includes a search bar, navigation menu, and introductory text about the program's purpose and standards.

Screenshot of the CCAC website showing the Experimental Animal User Training Core Topics Modules page. The page features the CCAC logo, a photo of a hand holding a mouse, and an introduction to the training modules.



Minimizing the Impact of the Infectious Disease on the Experimental Animal's Welfare

- Provision of relief from anxiety, distress, pain and suffering – Balancing welfare and science

Stress / Distress, resulting from: infections, surgical trauma, manipulations, anxiety, pain, suffering

Immune Function and Responses

Relief of Stress / Distress, through use of: anaesthetics, anxiolytics, sedatives, analgesics



Minimizing the Impact of the Infectious Disease on the Experimental Animal's Welfare

- Provision of physiological support i.e. fluids, nutrition

Fluids

40-80ml/kg/day, oral or parenteral

Supplemental nutrition

palatability, consistency, "treats"



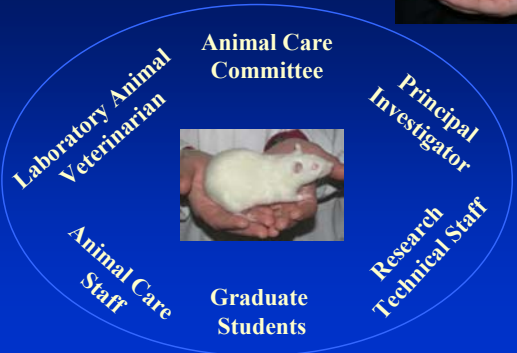
Minimizing the Impact of the Infectious Disease on the Experimental Animal's Welfare

- Provision of comfortable surroundings
- Conditioning prior to start of experiments
- All aspects of environmental enrichment • , e.g.,
 - soft, deep bedding
 - warmth
 - hiding places in cage
 - etc.
- consistent application across treatment groups



Minimizing the Impact of the Infectious Disease on the Experimental Animal's Welfare

- Treatment of the infectious disease



“... deciding which is the most suitable course to adopt when it is found necessary to ‘smooth the path to death’.”