2011 Summer Externs

Extern: Tara Martin, ASLAP Foundation Summer Fellowship

- Currently a student of Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM), Blacksburg, VA to graduate in 2013.
- Involved with clubs such as ASLAP, Student Member of the American Association of Public Health Veterinarians Food Animal Practitioners Club, Public Veterinary Practice Club, SCAVMA, Student Member, Maryland Veterinary Medical Association Student Member, Virginia Veterinary Medical Association, Member, Student Chapter Veterinary Emergency and Critical Care Society Pathology Club, VMRCVM

Mentor: Judy Hickman-Davis, DVM, PhD, DACLAM, Director, Laboratory Animal Training Program, ULAR; Associate Professor, Clinical Veterinary Preventive Medicine

Dr Hickman-Davis is an Attending Veterinarian for OSU facilities and as the Director; Quality Assurance Lab Large ensures the health of the animals used in research at OSU. She is the Director for the Laboratory Animal Residency Program. She is a member of ASLAP and AALAS, serves on the IACUC at OSU.

Research Project: “Effects of Air Flow on Ammonia Levels in IVC Rat Cages”
Effects of Air Flow on Ammonia Levels in IVC Rat Cages

Tara Martin
2011 ULAR Summer Extern

IVC Cages
- IVC cages are meant to provide filtered air flow directly into each cage.
- They protect rodents from exposure to environmental contaminants and pathogens.
- Animals do not have direct access to room air, so ventilation is important to prevent build-up of CO₂ and ammonia.

Tecniplast Cages
- ULAR uses Tecniplast cages to house rats.
- These cages have an inlet valve for clean air and an outlet valve for contaminated air.
- To reach the cage, valves must push through self-sealing ports in the back of each cage lid.

Intact Valves

Damaged and Occluded Valves
- Rat cages were found with condensation along the walls and lids.
- Some cages had broken or chewed valves.
- Other racks were found to have air valves clogged with debris.

Damaged Valves
- Damaged valves cannot push the self-sealing cage ports open, compromising airflow.
Occluded Valves

The manual for these racks provided by Tecniplast has no recommendation for removing air valves when sanitizing or cleaning the rack.

ULAR SOPs previously required racks to be sanitized every 6 months without removing valves. This SOP has been revised.

Current ULAR SOP

- Valves are removed from the rack and soaked in a bucket of 10% bleach solution for 10 minutes.
- Valves are rinsed, placed in a wire basket, and run through a rack or tunnel washer.
- Nozzles are checked after washing to be sure they are clean.
  - If not clean, valves are soaked again and re-washed.

Our Project - Purpose

- To see whether occlusion of valves affects the build-up of ammonia in Tecniplast Sealsafe IVC cages.
- To see how other institutions using Tecniplast Sealsafe caging systems clean their racks.

Hypothesis

- Cages with occluded air valves will have a faster increase in ammonia levels versus cages with valves that are not occluded.
- Cages with occluded air valves will reach higher overall ammonia levels than cages with valves that are not occluded.

Materials and Methods

- 16 rats were housed individually in Tecniplast cages in four groups:
  - Fully Open Valves: Both inlet and outlet valves were open and non-occluded.
  - Fully Occluded Valves: Both valves were artificially occluded. This was done by removing both the inlet and outlet valves from the rack.
  - Outlet Only: The fresh air inlet valve was artificially occluded by removing it from the rack.
  - Inlet only: The outlet valve was artificially occluded by removing it from the rack.
- Ammonia was measured every day using pHydron strips held 1 – 2” above bedding level.
  - This was done for 14 days or until ammonia levels reached above 25 ppm.
  - Two trials were performed – rats were shifted between groups to keep weights approximately equal across all groups.
Results

Average Days In Study

<table>
<thead>
<tr>
<th>Condition</th>
<th>Days</th>
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</thead>
<tbody>
<tr>
<td>100% Occluded</td>
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</tr>
<tr>
<td>100% Open</td>
<td>3</td>
</tr>
<tr>
<td>Outlet Only</td>
<td>6</td>
</tr>
<tr>
<td>Inlet Only</td>
<td>8</td>
</tr>
</tbody>
</table>

Results

Average Ammonia Per Day

<table>
<thead>
<tr>
<th>Condition</th>
<th>Ammonia ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Occluded</td>
<td>*</td>
</tr>
<tr>
<td>100% Open</td>
<td></td>
</tr>
<tr>
<td>50% Occluded</td>
<td></td>
</tr>
<tr>
<td>Outlet Only</td>
<td></td>
</tr>
<tr>
<td>Inlet Only</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions

• Cages with fully occluded valves reached harmful ammonia levels much more quickly than cages where inlet, outlet, or inlet and outlet valves are open.

• Air inlet and outlet valves should be routinely removed from the rack and checked to be sure they are not damaged or occluded with debris.

• Unnoticed occlusion could lead to a rapid build-up in ammonia to harmful levels.

• Air inlet and outlet valves should be cleaned routinely to be certain that air flows through cages appropriately.

• Tecniplast cages should not be used without cage wires to prevent damage to air inlet and outlet valves.

Survey

• A survey has been sent out to other facilities using Tecniplast cages.

• Questions include:
  ◦ Cleaning protocols for Tecniplast racks
  ◦ Type of bedding and food used in each facility
  ◦ Number of rats housed in Tecniplast IVC cages at each facility

• Goal is to find out whether other facilities have had similar issues with caging and to see what their cleaning protocols are like.

Rat Rack Cultures

Staph/Strep sp.

Pre-rack/valve washing culture swab
Rat Rack Cultures

Post-rack /valve washing culture swab

No Growth!!
**Extern: Ellen Scherer, Cass Summer Fellowship**

- Currently a student at The Ohio State University, College of Veterinary Medicine to graduate in 2014
- Involved with clubs such as: SCAVMA, Radiology Club, ASLAP, OVMA and Omega Tau Sigma.

**Mentor:** Dr Stephanie Lewis, DVM, MS, DACLAM. Director, Large Animal Clinical Medicine, University Laboratory Animal Resources; Assistant Professor, Clinical. Veterinary Preventative Medicine

Dr Lewis is Attending Veterinarian for OSU facilities and as the Director of Large Animal Clinical Medicine provides clinical and didactic support for the Laboratory Animal Residency Program. She is a member of ASLAP, ACLAD and AALAS, serves on the IACUC at OSU and at the Veterinary Institute at Bradford.

**Research Project:** Evaluation of Various Routes of Administration of Meloxicam for Postoperative Analgesia in Mice.
Evaluation of Various Routes of Administration of Meloxicam for Postoperative Analgesia in Mice

Ellen Scherer
Summer 2011

Non-Steroidal Anti-Inflammatory Drugs

- Commonly Used For:
  - Fever
  - Arthritis
  - Analgesia
  - Mild to moderate
- Other uses:
  - Endotoxemia
  - Anticoagulant activity
  - PDA closure

NSAID - pharmicokinetics

- Metabolized in the Liver
- Excreted by kidneys and in feces
- High protein binding affinity
  - Liver metabolism is limited by enzyme capacity, not blood flow
  - Dosing varies by species
  - High potential for drug interactions

NSAID - Goal...

- Inhibit the enzyme cyclooxygenase

NSAID - side effects

- GI Ulceration
- Impaired platelet aggregation
- Delayed parturition
- Blood dyscrasias
- Impaired bone healing
- Nephrotoxicity (horses)

NSAID - Side Effect Cont.

- Allergies
- Reproductive system
- Male fertility?
**COX 1 & 2**

- The COX enzyme catalyzes arachidonic acid to form prostaglandins and leukotrienes
- Activation site is at the end of a long narrow hydrophobic “tunnel or channel”
- Heme-dependent peroxidase activity is required for COX activity
- COX 1 & 2 have different activation switches

**COX 1**

- Constitutive Production
- Concentration with in the body remains stable
- Carries out normal physiologic production of prostaglandins
- Attachment site is smaller compared to COX 2 attachment site

**COX 2**

- Induced
  - Not normally present
  - Responsible for production of prostaglandins in inflammatory responses

**COX in the Inflammatory Process**

Inflammatory Stimuli (disease/trauma) -->
Membrane phospholipids release
Arachidonic Acid -->
Cyclooxygenase catalyzes arachidonic acid to prostaglandins & leukotrienes -->
Prostaglandins create inflammatory response

**Aspirin Specifically**

- Aspirin is the only NSAID that covalently modifies a residue in the “tunnel” of the COX compound. Thus, aspirin irreversibly inactivates both COX 1 & 2.
**Tylenol - Acetaminophen**
- Synthetic
- Produces analgesia & antipyresis
- Does NOT possess significant anti-inflammatory activity
- ~25% protein bound
- Contraindicated in cats
- Excreted in milk

**Tylenol - Drug Interactions**
- Can potentiate the effects of coumarin or inandione anticoagulants
- Doxorubicin
  - This leads to hepatotoxicity

**Ibuprofen**
- Anti-inflammatory, analgesic, & antipyretic activities
- Propionic acid derivative
- Non-specific COX inhibitor
- ~90% bioavailability in oral dosing of dogs
- Highly plasma protein bound
- Eliminated in feces and urine

**Ibuprofen - Side Effects & Drug Interactions**
- GI ulceration
- Blood thinning
- At high doses can cause renal effects
- Decrease in efficacy of blood pressure lowering drugs
- Interferes with the secretion of lithium & aminoglycosides

**Ibuprofen - Dosing**
- Rabbits:
  - 2.2 mg/kg PO
- Rats:
  - 5 mg/kg SC or 5-10 mg/kg PO
- Chinchillas:
  - 4 mg/kg SC

**Meloxicam**
- "New" NSAID
- Selective inhibition of COX 2
- Analgesic, anti-inflammatory, antipyretic
- ~97% protein bound
- Metabolites are excreted in the feces
- Significant enterohepatic recirculation
### Meloxicam - Half-life

- **Species specific:**
  - Dogs = 24 hrs
  - Pigs = 4 hrs
  - Horses = 3 hrs
  - Cattle = 13 hrs

### Meloxicam - Drug Interactions

- Drug interactions occur by displacing other drugs, or by being displaced by other drugs with high protein binding affinity.
- If used with other drugs that alter hemostasis and/or cause GI erosion there is an increased likelihood of bleeding or ulceration.
- May antagonize the antihypertensive effects of ACE inhibitors.

### Medicine in the Water Bottles: Pros

- Minimal animal restraint and distress
- Less opportunity for administration complications
- Antibiotics are available for administration in the drinking water for poultry and livestock and their effectiveness and stability has been evaluated.

### Medicine in the Water Bottles: Cons

- Compounding the drug influences:
  - Water type
  - Dilution used
- May compromise chemical stability, purity, & potency of active ingredient.
- Other Factors:
  - Water temperature
  - pH
  - Chemical properties Reactivity
  - Light.

### Journal: JAASLAS 39 (6)

- An evaluation of Analgesic Regimens for Abdominal Surgery in Mice
  - In this study mice were given acetaminophen, acetaminophen-buprenorphine combo, or ibuprofen in the water bottle 1 day prior to surgery and continued there after.
  - First 2 days post-surgery water consumption fell.

### Journal: JAASLAS 46 (5), 26-32

- Amoxicillin - Clavulanic Acid and Trimethoprim-Sulfamethoxazole In Rodent Feed and Water: Effects of Compounding on Antibiotic Stability
  - Amoxicillin & clavulanic acid concentrations in water bottles at extended times post mixing
  - Used different types of water.
Expected, Initial Concentrations

- Amoxicillin --> 0.96 mg/ml
- Clavulanic Acid --> 0.14 mg/ml

Actual Concentrations: Over 7 day period

- Amoxicillin
  - RO water --> 1.18 - 1.29 mg/ml
  - Tap water --> 1.09 - 1.22 mg/ml
  - Acid water --> 0.43 - 0.50 mg/ml

- Clavulanic Acid
  - RO water - statistically significant time-dependent decrease in concentration beginning at 72 hrs
  - Tap water --> 0.02 - 0.04 mg/ml
  - Acid water - none detectable at any time point

Lack of Analgesic Efficacy in Female Rats of the Commonly Recommended Oral Dose of Buprenorphine

"An oral dose approximately 10 times higher than that recommended was necessary to induce a level of analgesia similar to that of the therapeutic gold standard."

Acetaminophen Self-Administered in the Drinking Water Increases the Pain Threshold of Rats (Rattus norvegicus)

- Pros
  - Reduces stress on animals
  - Less staff time

- Cons
  - Neophobia

Used 32 mg/ml cherry flavored acetaminophen in water

Findings:

- Initial neophobia to acetaminophen water & rats did not drink much of it as tap water
- Rats that did undergo surgery did drink more acetaminophen water than unmanipulated animals

Evaluation of Various Routes of Administration of Meloxicam for Postoperative Analgesia in Mice

- The proposed project investigates the use of the non-steroidal anti-inflammatory (NSAID) agent meloxicam as a suitable single agent for postoperative analgesia in mice.
- Evaluate the administration of the injectable formulation of Meloxicam via subcutaneous (SC) injection or in the drinking water, or the oral suspension in drinking water following laparotomy surgery in C57Bl6 mice.
### Evaluation of Various Routes of Administration of Meloxicam for Postoperative Analgesia in Mice

#### Protocol Activities
- Survival laprotomy surgery
- Food and Water monitoring
- Behavioral Testing
- Hot Plate Test

### Resources
- [An Evaluation of Analgesic Regimens for Abdominal Surgery in Mice](http://cat.middlbury.edu/~chem/chemistry/class/bioch314/presentations/diabetes.html)
- [Acetaminophen Self-Administered in the Drinking Water Increases the Pain Threshold of Rats (Rattus norvegicus)](http://cat.middlebury.edu/~chem/chemistry/class/bioch314/presentations/diabetes.html)
- [Lack of Analgesic Efficacy in Female Rats of the Commonly Recommended Oral Dose of Buprenorphine](http://cat.middlebury.edu/~chem/chemistry/class/bioch314/presentations/diabetes.html)
- [An Evaluation of the Analgesic Regimens for Abdominal Surgery in Mice](http://cat.middlebury.edu/~chem/chemistry/class/bioch314/presentations/diabetes.html)
- Judy Hickman-Davis, DVM, PhD, DACLAM