American Academy of Veterinary Pharmacology and Therapeutics

21\textsuperscript{th} Biennial Symposium

“New Ideas, New Voices”

Overland Park Convention Center, Overland Park, Kansas
August 23-26, 2019
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21st Biennial of the American Academy of Veterinary Pharmacology and Therapeutics
“New Ideas, New Voices”
Overland Park Convention Center, Overland Park, Kansas
Scheduled Program

Friday, August 23, 2019
5:00-7:00 pm  Registration and Reception

Saturday, August 24, 2019
7:00-  Nametag and ticket pickup
7:00-8:30  ACVCP Business Meeting
8:45-9:00  Welcome to the Biennial, Rob Hunter

TOM POWERS MEMORIAL KEYNOTE
9:00-9:45  Change is Coming: New Therapies, Big Data, New Capital for New Companies. Thoughts on the Future, Linda Rhodes

9:45-10:00  Break

SESSION 1: New Ideas, New Voices
10:00-10:45  Drug Testing in Livestock Show Animals, Travis Mays
10:45-11:30  Novel Uses of In Vivo Ultrafiltration for Pre-clinical Pharmacokinetic Studies in Animals, Kristin Messenger
11:30-12:15  Applications of Metabolomics in Veterinary Pharmacology Research: Advantages, Limitations and Future Perspectives, Nicolas Villarino
12:15-1:15  Lunch
1:15-2:00  Improving the Effectiveness of Tramadol in Dogs through Understanding CYP Metabolism, Tania Perez Jimenez

SESSION 2: Graduate Student Presentations
[These students are eligible for the AAVPT/ACVCP Resident/Graduate Student Research Award.]
2:00-2:15  Pharmacokinetics and impact of nonsteroidal anti-inflammatory drugs in biomarkers of pain and inflammation at piglet castration, Emma Nixon
2:15-2:30 Evaluation of Multiple Doses of a Long-acting Oral Opioid Containing an Abuse Deterrent in Dogs, Ally Fitzgerald

2:30-2:45 Characterization of CYP450 mediated metabolism of the polymorphic CYP2D6 probe drug codeine in horses, Sophie R. Gretler

2:45-3:00 Break

3:00-3:15 Canine albumin and orosomucoid (α1-acid glycoprotein) polymorphisms and their impact on drug plasma protein binding, Ana P. Costa

3:15-3:30 Metabolism, pharmacokinetics and selected pharmacodynamic effects of codeine following a single oral administration to horses, Sophie R. Gretler

3:30-3:45 Clinical Efficacy of an Oral Long-Acting Analgesic with a Human Abuse Deterrent in Perioperative Dogs, Ally Fitzgerald

3:45-4:00 Equine UDP Glucuronosyltransferase 1A1, 2A1, 2B4, 2B31: cDNA cloning, expression, and initial characterization, Briana D. Hamamoto-Hardman

4:00-4:15 Practical and affordable tick prevention in horses in Grenada, West Indies, Inga Karasek

4:15-5:30 Break and Poster set-up session

5:30-7 Wine and cheese reception/Poster session

7:00-9:00 Banquet and Silent Auction

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Sunday, August 25, 2019

7:00-8:30 am AAVPT Business Meeting

8:45-9:00 am Introduction to the day, Rob Hunter

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LLOYD E. DAVIS AWARD LECTURE

9:00-9:45 KLM on ABC transporters: A retrospective analysis of cofactors, Katrina Mealey

9:45-10:00 Break

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SESSION 3: Antimicrobials and Diagnostic Labs

10:00-10:45 Breakpoint Setting History and Industry Perspective, Mark Papich

10:45-11:30 Diagnostic Labs and Breakpoints, Dubra Diaz-Campos

11:30-12:15 VET09 – Guidance on Interpreting, Antimicrobial Susceptibility Reports, Virginia Fajt
12:30-1:30  Lunch

SESSION 4: Graduate Skills and Asilomar Update

1:30-1:35 pm  Introduction to the Afternoon Session, Rob Hunter

1:35-2:05  Training Veterinary Pharmacologists - Updating the Blueprint for Graduate Education, Virginia Fajt

2:05-2:35  Industry Thoughts and Needs, Dan Keil

2:35-3:05  Industry’s Unmet Needs, Paul Cassady

3:05-3:20  AAVPT Long Range Planning Task Force

3:20-3:35  Break

3:35-4:05  Board Certification in the Current World, Mike Apley

4:05-4:35  Breakout Discussion on Graduate Training

4:35-5:15  Feedback from Breakout Discussion and Panel Q&A

Monday, August 26, 2019

SESSION 5: Start-ups and New Business Ideas

8:45-9:00 am  Introduction to Session, Rob Hunter

9:00-9:30  Intellectual Property, Michael Annis

9:30-10:00  I have this idea, now what? Hank Mills

10:00-10:30  Where does the money come from? Shawn Glinter

10:30-11:00  Transferring Tech out of a University, Troy Brady

11:00-11:30  Transferring Tech into a Company, Scott Brown

11:30-12:00  Q&A Panel and Conference Wrap-up
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**Tom Powers Memorial Keynote Address**

**Change is Coming: New Therapies, Big Data, New Capital for New Companies: Some Thoughts on the Future**

Linda Rhodes, VMD, PhD

**Introduction**

The animal health industry has changed dramatically over the last ten years, and is poised for an acceleration of change. Our customers are changing, as practices consolidate into corporations, and sales that used to be direct to veterinarians are going direct to consumer, via a robust internet market. Companies are breaking away from their human health ‘parents’ and becoming stand alone public companies, as Zoetis and then Elanco have done, with likely more to follow. Innovative therapeutic options, beyond small molecule drugs, including a role for diagnostics, genomics and big data are being explored for uses in both production and companion animals, as slowly an ‘animal biotech’ ecosystem is emerging. This talk will highlight some examples, and discuss some of the opportunities for innovation in animal health.

**The Big Picture**

Human health went through the biotech revolution when Genetech was founded in 1976, and AmGen in 1980. These start ups formed the basis of the biotechnology industry, a factory for innovation that both developed their own products and fed the large pharmaceutical companies innovative new ideas. Their success encouraged the funding of thousands of companies focusing on human health innovation over the last forty years.

In animal health, we did not have this ecosystem for many years, but now, with the advent of Aratana Therapeutics founded in 2011, and a handful of other small companies, venture capital has started investing in start-ups focused on animal health. Many new companies are being formed each year, and some capital is now available, as the investment community begins to understand the value and risk/reward profile of animal health.

This influx of capital and interest has resulted in some exciting new approaches to find solutions in for unmet needs in animal health. In addition, the legacy companies are investing in innovation as they get ready for transformative change. A few of the areas where the pace of change is accelerating include:

- Monoclonal antibodies
- Gene delivery
- Alternatives to antibiotics
- Data and artificial intelligence

**Monoclonal Antibodies**

Many highly successful monoclonal antibodies have been developed in human health, with the first licensed in 1986 for preventing kidney transplant rejection and since then over 80 have been approved for a wide variety of indications. In animal health, it was widely thought that cost of goods of monoclonal antibodies was too expensive to be commercially viable, and companies were reluctant to be the first through the regulatory gauntlet. With the dramatic commercial success of Cytopoint (anti-IL31), first approved in 2016 for atopic dermatitis in dogs, with sales of over $100M in 2018, a new companies have been formed to develop monoclonal antibodies for pets.
**PetMedix**, a Cambridge UK based company, recently raised $20M in a Series A financing to develop monoclonal therapeutics for dogs and cats. They have not publicly released their targets but have published in depth work describing the canine antigen receptor loci (1) as the basis for their technology for developing canine Mabs.

**NexVet Biopharma** developed monoclonal antibody therapeutics for the alleviation of pain in dogs and cats (anti-nerve growth factor) and was in the process of conducting studies for regulatory approval when the company was acquired by Zoetis (2).

**Adivo**, a company that was spun out of the human biotechnology firm Morphosys in 2018, recently signed a licensing deal with Bayer Animal Health. They are selecting species-specific antibodies using a fully canine phage display library, looking at companion animal targets defined by Bayer. They received their seed investment from a German venture capital group (High-Tech Grunderfonds), a Swiss group (Occident Group) and from MorphoSys.

**Gene Delivery**

Gene delivery, or gene therapy, is the process by which a piece of DNA encoding a specific protein is administered, most commonly using a viral vector, such as adeno-associated virus (AAV). The type of AAV may determine which tissue the genetic material lodges in, but whatever the tissue, the protein it codes for is produced constitutively, potential for the lifetime of the individual. Much of the original work of gene therapy was done at the University of Pennsylvania, with the goal of treating children with genetic mutations causing loss of function, such as immune deficiencies. Work done in dogs with hemophilia showed that a single injection with an AAV bearing the gene for the missing clotting factor could restore these dogs to normal for their entire lifetime (3).

Gene delivery is now a major focus of dozens of biotechnology companies developing therapies for serious and life threatening rare genetic deficiencies in humans. The FDA issued its first approval of an AAV vector for treatment of congenital blindness in 2017 (4).

Again, animal health is far behind human health, and likely again, it may be due to worry about cost of goods and being first on what is likely to be a difficult regulatory path. An important consideration in selecting potential targets is that the treatment is, in fact, lifetime, and once injected cannot be ‘turned off’ or modulated. Two companies have been founded to develop gene therapy for companion animals.

**ScoutBio** is a company founded by Dr. Jim Wilson, at University of Pennsylvania, who has been at the forefront of gene therapy for humans. They are developing an AAV-erythropoietin treatment for anemia due to chronic kidney disease in cats. Theoretically, after a single injection, erythropoietin deficiency would be corrected for the lifetime of the cat. A clinical trial is currently in progress.

**Panion Animal Health** is developing a new treatment for epilepsy in dogs using an AAV expressing neuropeptide Y and receptor peptide Y2 to treat drug refractory idiopathic epilepsy via intracranial injection. Although this method of treatment is challenging, it would likely need to be given only once for a lifetime effect.
Cambridge Animal Health was started based on observations in Dr. Bruce Hay’s laboratory at Cal Tech that mice injected with AAV virus delivering a gene for a monoclonal antibody against gonadotropin releasing hormone (GnRH) were sterile (5). This technology could conceivably be developed as a single injection, permanent sterilant as an alternative to surgical spay/neuter. To be successful, a high affinity canine or feline specific anti-GnRH monoclonal antibody would need to be identified. If a single injection lifetime effect could be achieved, this could be a breakthrough tool to control overpopulation in cats and dogs.

Alternatives to Antibiotics

Because of the growing concern about use of antibiotics in animals causing resistance, there is an urgency to develop alternatives, including prevention with new vaccines, manipulation of the microbiome, and the use of bacteriophage. A variety of companies have been founded to explore these possibilities.

- **AgBiome, Inc.** has a collaboration with Elanco to develop products for swine that they describe as “innovative probiotic solutions” for “gut health”. No other details are public.
- **Evonick Industries** partnered with Perdue AgriBusiness to develop what they term a ‘gut care’ probiotic (*Bacillus subtilis DSM32315*) as a granular feed additive to create a “balanced gut microbiome in poultry and monogastric animals”.
- **Anizome** is working to identify new probiotics for a variety of companion animal diseases.
- **Proteon Pharmaceuticals** is developing bacteriophage-based products for mastitis in dairy cows, and *E. coli* in poultry. They have developed Bafasal® to eliminate Salmonella in poultry and Bafador® to treat *Pseudomonas* and *Aeromonas* in aquaculture.
- **Lysando** is working to develop alternatives to antibiotics using their proprietary drug discovery platform from bioactive peptides optimized from bacteriophage. The products are progressing in species in early safety and efficacy studies for dermatology/topical indications.
- **Resilient Biotics** is developing “microbiome derived therapies” for animal health targeting bovine respiratory disease, with “live, microbial therapeutics”. They claim “this is a novel way to treat BRD via restoration of a healthy respiratory tract microbiome. Our treatment is based on extensive analysis of microbial dysbiosis during disease onset, and we have designed a therapeutic cocktail to exclude opportunistic pathogens and promote beneficial host immune response.”
- **BioPlx** is a company that “maps functional intra-biome relationships and uses high capacity computational biomics to extract global data sets”. They are developing “specialized organisms with known attributes to occupy specific biome niches”. The company has focused on human health but is now looking for a partner to help them develop a product for bovine mastitis.

Big Data

Both **Resilient Biotics** and **BioPlx** are using “big data” and machine learning, also called artificial intelligence, to sort through microbial genomes and identify target microbes and relationships among microbes. Although information on the technologies these companies are using is scarce, it appears their approach is to identify non-pathogenic microbes that can colonize particular spaces in the body, such as gut or lung, and likely even more granular, a particular gut section, or the area of the respiratory system, like the bronchi. These newly established microbes may be able to modulate the immune system, or
alternatively occupy a niche to exclude pathogens. These approaches are made possible by the advances in computational biology and artificial intelligence.

**Nuritas**, a company that has built a predictive algorithm to source novel peptides and proteins as nutraceuticals and APIs from natural sources is launching a new company to find new products for both companion and production animals.

Animal health companies are aware that developing expertise in big data and artificial intelligence will be important for their future. In production animals, there are approaches to animal monitoring, that can help producers. For example, systems can detect when a cow is in heat by tracking her steps, and whether pigs are developing respiratory disease by monitoring coughing in the barn. Eventually, these basic data gathering and analysis systems will expand to include diagnostics and even genetics to help farmers better manage their animals. Merck purchased **Antelliq** in 2018 and talked about the company’s expertise in animal identification, animal monitoring and smart data management for both production and companion animals. Previously, Zoetis had purchased **Smartbow** a technology for monitoring dairy cows, including rumination, heat detection and cow location.

**Regulations**

For new technologies and approaches to make it to market, we also may need new regulatory frameworks. The first gene therapy, the first microbiome product that is a mix of novel bacteria, the first bacteriophage product, will all be new challenges for regulators in understanding how to evaluate safety, and novel manufacturing technologies. Particularly in the area of production animals, where the need for antibiotic alternatives is greatest, the traditional approach to evaluating human food safety will likely not be applicable, and companies and regulators will have to work together to explore science-based alternatives.

**New Capital**

Start up companies developing these new opportunities require capital, and slowly both venture capital companies and private equity funders have a growing awareness of the commercial opportunities in animal health. In addition, there has been a marked increase in the interest of human biotech companies to explore the application of their technologies to animal health unmet needs. This is a welcome change and should encourage an increased pace of innovation in our industry.

**Conclusions**

The pace of change in the animal health industry has accelerated, fueled by the clear need for innovation for our industry, the demonstration of commercial success of new innovative products, such as Galliprant, Apoquel and Cytopoint, all first in class therapies, and the increasing interest of the human biotech companies in animal health applications of their technologies. Our challenge is to continue to demonstrate the path from new science to commercial product is achievable at a reasonable return on investment. Big data will transform our product discovery and development in ways we cannot yet anticipate and represent a fresh and exciting approach. Our challenge is to be open to change, and rigorous in selection of the best science to support new solutions to unmet needs.
References
Testing for drugs that have the ability to enhance performance in competition animals is common practice. For example, drug testing is performed in many different animal competitions that include: Quarter Horse, Thoroughbred, and Standardbred (harness) racing; equestrian events including dressage, jumping, draft horse showing, trail riding, cutting, and reigning; greyhound racing; camel racing; pigeon racing; and dog pulling/sledding. Another animal industry that utilizes drug testing is the livestock show industry. Similar to other performance animal competitions, livestock shows utilize drug testing to foster fair competition among competitors and ensure animal welfare. Ante-mortem samples, such as urine, blood, and feces are tested for a variety of drugs that are considered to have the ability to enhance performance in livestock. These drugs include nonsteroidal anti-inflammatory drugs (NSAIDs), beta-adrenergic agonist drugs with repartitioning effects, analgesics, stimulants, sedatives and tranquilizers, antihistamines, and illicit drugs.

Testing livestock exhibited at shows for drugs is more complex than the other performance animal competitions, however. Unlike other performance animal competitions, a rationale for drug testing of exhibited livestock is to ensure food safety. In addition to testing ante-mortem samples for performance-enhancing drugs, post-mortem samples from exhibited livestock also are tested to protect the food supply. Once a livestock project is completed, the animal will enter the food supply. Exhibition animals that enter the food supply represent a large number of carcasses across the country. In 2004 in Texas alone, it is estimated that more than 14 million pounds of carcasses from livestock projects entered the food supply (Anonymous, 2016). Post-mortem samples, such as liver, kidney, and muscle are screened for a variety of drugs that include NSAIDs, beta-adrenergic agonists with repartitioning effects, and antibiotics.

Also unique to livestock shows compared to other performance animal competitions is how drug testing is regulated. Regulation of drugs detected in post-mortem samples from exhibition animals is under the purview of the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS). The FDA approves tolerances and withdrawal times for meat and other edible tissue as part of approval of drugs for use in food animals. Regulation of drugs detected in ante-mortem samples from exhibition animals is under the purview of the board of directors of the individual livestock show or fair, and usually involves a so-called “zero tolerance” policy (Anonymous, 2018; Anonymous, 2018). Regulation of drugs detected in ante-mortem samples can be divided into 2 categories: 1) prohibited substances, and 2) drugs that are administered in a legal manner. While “zero tolerance” is appropriate for prohibited substances, thresholds and decision limits should be established for drugs administered legally.

Establishing decision limits for drugs administered legally to exhibition animals, such as therapeutic drugs like NSAIDs, is a novel approach for regulating drug testing of ante-mortem samples in the livestock show industry. Determination of decision limits should be based on pharmacokinetics and pharmacodynamics. Establishing a numerical decision-point to classify a sample as being negative or positive also must account for measurement error, and intra- and inter-individual variance. Currently, the difference between a sample testing positive or negative is determined by the limit of detection of the analytical assay. In other words, if the analytical instrument can detect the presence of a drug in a
sample, the sample is deemed positive. Changes in analytical testing methods in recent years have lowered the limit of detection for drugs used in a legal manner. This has increased the number of positive tests, raising concerns about the policy of “zero tolerance”.

Detection of trace levels of therapeutic drugs in animals exhibited at livestock shows raises 2 main concerns. The first concern relates to the degree to which very low levels of therapeutic drugs provide a performance-enhancing effect in the animal. Animals exhibited at livestock shows are unique compared to other performance animals, as they compete based on appearance rather than physical activity. Therefore, drugs used in exhibition animals to enhance performance do so by altering appearance, such as masking pain, lameness, or inflammation, and improving leanness and muscling. In addition to lack of ability to detect physical effects of drugs in the context of exhibited livestock, researchers have demonstrated that untreated animals can have detectable levels of therapeutic drugs, like flunixin, from exposure to the drug in the environment. Comingling of treated and untreated horses (Barker, 2008) and pigs (Hairgrove, Mask et al., 2019) resulted in detectable levels of drugs like flunixin in the untreated animals.

The second concern with “zero tolerance” when regulating therapeutic drugs that are legally administered is predicting a withdrawal time in urine or plasma to avoid detection of drug. Veterinarians who administer therapeutic drugs to exhibition animals in a legal manner face uncertainty when estimating the appropriate amount of time for drug elimination prior to competition. If exhibition animals are not to be deprived of proper veterinary care, suitable information on the time after administration that therapeutic agents may be detected must be made available to the veterinary profession.

Three different methods for establishing decision limits for therapeutic drugs detected in animals exhibited at livestock shows were evaluated. The first method considered for establishing decision limits in therapeutic drugs detected in animals exhibited at livestock shows was the 95/95 Tolerance Interval (Products, 2000) used in the US by the Racing Medication & Testing Consortium (RMTC) Scientific Advisory Committee (SAC). The second method was the irrelevant plasma concentration (IPC) and irrelevant urine concentration (IUC) approach used by regulators in horse racing in Europe (Toutain & Lassourd, 2002). The third method was a novel method using pharmacodynamic models and incorporating variability and measurement error. This new approach could be used to define thresholds for identifying potentially unacceptable levels of therapeutic drugs in urine from exhibition animals as it takes into consideration drug effects and multiple aspects of measurement error and population variability.

References
Products, E.A.f.t.E.o.M. (2000). *Note for guidance for the determination of withdrawal periods for milk*

NOVEL USES OF IN VIVO ULTRAFILTRATION FOR PRE-CLINICAL PHARMACOKINETIC STUDIES IN ANIMALS

Kristen Messenger, North Carolina State University

There have been several methods to study the tissue concentrations and distribution of drugs, including blood/plasma sampling, tissue homogenates, tissue cages, microdialysis, ultrafiltration, bronchoalveolar lavage, and skin blister fluid, to name a few. Each of these techniques has unique advantages and disadvantages which have been previously reviewed (Deitchman & Derendorf; 2014). Whole blood, serum, or plasma sampling and analysis are convenient and common methods used in PK-PD studies, thanks to ease of sampling technique, drug analysis, and understanding of results. However, a major disadvantage to this technique is that concentrations in plasma do not reflect those at the site of action (Toutain et al., 2001; Deitchman & Derendorf, 2014).

Microdialysis (MD) is one of the most commonly used methods to collect local, protein-unbound drug concentrations (Muller et al., 2004). Advantages of MD are many, and include the collection of unbound, pharmacologically active drug, continuous sampling, and ability to sample from a variety of tissue sites. Placement of MD probes is minimally invasive and does not cause permanent damage to the patient. Disadvantages include complicated calculations to determine recovery, expensive pumps and equipment, requirement that subjects be confined or immobile, and low volume collection of samples. The last of these is a limitation in that there may not be enough sample for simultaneous determination of drug concentrations and pharmacodynamic endpoints such as inflammatory biomarker quantification.

*In vivo* ultrafiltration is a technique very similar to MD in concept, but has important differences that can be considered advantages. This method involves the placement of an ultrafiltration probe into the tissue of interest. Placement is minimally invasive and is performed using local anesthetic and an introducer needle, depending on the species and tractability (see cartoon figure below).

The probe consists of 3 loops made of a semi-permeable dialysis membrane made of polyacrylnitrile, with a molecular weight cut-off of 30,000 Daltons. The specific molecular weight cut-off allows for the exclusion of large proteins and peptides, such as blood and albumin (MW approximately 60,000 Daltons). Tissue fluid is collected through the ethylene propylene tubing using a vacuum collection system (see cartoon of dog, below), which allows for continuous fluid sampling at a rate of approximately 100 µL/hr,
although sampling rates and volume will depend on strength of the vacuum and hydration status of the animal or tissue where the probe is located.

Figure (above): Graphic of an in vivo ultrafiltration probe, vacutainer collection assembly, and placement in a dog. From http://www.basinc.com/mans/uf.pdf

Multiple samples over time can be obtained with minimal disturbance to the animal, and the probes can be left in place for several days with no adverse consequences. The probes are made of non-reactive polymers, thereby causing minimal inflammatory responses and can be left in an animal for several days without any adverse effects (Linhares & Kissinger, 1993). In fact, our laboratory has left ultrafiltration probes in place for up to 14 days in some animals (cattle).

Other advantages of UF over MD include the absence of added perfusate, which simplifies calculations including the initial analyte recovery. Also, depending on the analyte of interest, less sensitive detection methods can be used since the analyte is not diluted by the perfusate. Lastly, this technique does not require the purchase and use of costly driver pumps.

Figures (above): Left: Scanning electron microscopy image of cut end of one loop of an unused UF probe, showing hollow fiber and smooth outer surface. Right panel: Scanning electron microscope image of outer surface of a used UF probe removed from an animal, demonstrating fibrin deposition on outer surface, mixed with inflammatory cells and red blood cells.
We have successfully combed in vivo ultrafiltration methodology with previously established inflammatory models (carrageenan) may to study both drug pharmacokinetics and pharmacodynamics directly at effect sites in the body (Messenger et al., 2016), and more recently in naturally occurring surgical models to study the anti-inflammatory effects of different analgesic drugs (Southern et al., 2019). Other investigators in our laboratory have applied this methodology to study antimicrobial concentrations in the GI tract of cattle (Warren et al., 2014; Foster et al., 2016; Ferguson et al., 2018) as well as in the teat cistern of dairy cattle (Mzyk et al., 2018). Our most recent work includes the use of ultrafiltration probes into different segments of the intestinal lumen to quantify locally-acting peptide drugs and their fragments once exposed to peptidases in the GI tract.

This presentation will review background, scientific and technical aspects of in vivo ultrafiltration, discuss troubleshooting, and lastly, will present some of the unique experiments where our laboratory has applied this methodology.

References


Additional Resources
- https://www.basinc.com/
APPLICATIONS OF METABOLOMICS IN VETERINARY PHARMACOLOGY RESEARCH: ADVANTAGES, LIMITATIONS AND FUTURE PERSPECTIVES

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Introduction
Inter-individual variability in drug response upon drug administration is caused by the interaction between drug pharmacology and patients’ (patho)physiological status. Individual variations in (patho)physiological status may result from, breed, genetic polymorphisms, age, sex, gut microbiota, disease-related factors, and environmental factors. Identification and quantification of predictors of inter-individual variability in drug pharmacology can expedite veterinary drug discovery. Also, predictors of individual responses to a particular drug can inform clinicians in decision-making process for drug selection and drug dosing regimens. Current research reveals that the metabolome can contain predictors of inter-individual differences in drug response.

Metabolome
All living organisms contain low-molecular-weight substances (LMWs; <1500 Da), such as amino acids, carbohydrates, fatty acids, organic compounds and peptides. The collection of LMWs in a biological sample is called metabolome. The metabolome of a biological sample can include the metabolic breakdown products from foods, drugs, endogenous waste metabolites, and bacterial end products. Identifying and quantifying a broad range of endogenous and exogenous LMWs allows the investigation of the phenotypic outcome resulting from complex interactions between genotype, lifestyle, diet, nutrition, environmental exposure, gut microflora and pharmacological interventions.

Metabolomics
The complete set of LMWs present in a biological system in a given physiological state at a given time point can be determined and studied using a metabolomics approach. Metabolomics is a multidisciplinary science, which combines sophisticated computational and statistical methods and analytical chemistry. Statistical analysis of metabolomics data entitles chemometrics, univariate and multivariate analysis. Statistical analysis of metabolomics data requires specialized mathematical, statistical and bioinformatics tools such as MetaCore™, MetaboAnalyst, InCroMAP, 3Omics and SIMCA. Detection of a broad range of LMWs requires special analytical platforms. The most common analytical platforms used for metabolomics analyses are LC/MS, GC/MS and NMR spectroscopy. Other alternatives platforms include capillary electrophoresis coupled to ESI-MS, LC with UV/visible absorbance, photodiode array or electrochemical detectors, Fourier-transform infrared spectroscopy (FT-IR). Fourier-transform ion cyclotron resonance mass spectrometry FTICR-MS and direct infusion-mass spectrometry (DI-MS). The metabolome is very diverse. Despite advances in the methodology and instrumentation, analytical methods able to detect the entire collection of LMWs in a biological sample remains to be developed. Hence, studies using a single analytical platform only capture a fraction of the metabolome.

Experimental strategies in metabolomics
The overall steps in a metabolomics workflow include a) biological question, b) experimental design, c) data acquisition, d) raw data preprocessing, e) statistical analysis, and f) biological interpretation. The metabolome is widely influenced by several and diverse factors such as environmental and lifestyle factors. Because metabolomics data is highly dependent on multiple factors, to conduct successful metabolomics studies, it is essential to control, identify, and eliminate the external factors that can affect the results. Sample collection, handling, and storage require special attention during the design of the experiment. Furthermore, a method of normalization to use, if any, needs to be defined a priori, for example, the use of creatinine for normalizing relative quantities of LMWs in urine.

Applications of metabolomics ion biomedical research
Since its first applications in the 1960s and 1970s, the use of metabolomics as a research tool has expanded into fields such as physiology, nutrition, infectious diseases, toxicology and pharmacology. One of the objectives of metabolomics is to define a metabolic signature or metabotype. A metabotype is a quantifiable readout of biochemical state from normal physiology to diverse pathophysiologies. Such metabolic signatures provide predictive, prognostic, diagnostic, and surrogate markers of diverse disease states; inform on underlying molecular mechanisms of diseases; allow for sub-classification of diseases, and stratification of patients based on metabolic pathways impacted; define a metabotype for each specific genotype, offering a functional read-out for genetic variants; provide a means to monitor response and recurrence of diseases, such as cancers and infectious diseases.

A recent study showed that genetically similar C57Black-6 mice from different commercial vendors had significant differences in parasite burden (~60% parasitemia in “susceptible” mice vs. ~10 % parasitemia “resistant” mice) and mortality after infection with multiple Plasmodium species. The study proved that this susceptibility was, in part, driven by differences in the gut bacterial community. The resistant and susceptible mice phenotype, was also correlated with the differences in the gut and plasma metabolome. Extrapolating results of this study to pre-clinical drug development, the inclusion of animals from a particular commercial vendor disregarding different susceptibilities to this parasite may have a profound impact on drug development programs. Results of this study suggest that metabolotypes could be used as a phenotypic tool to stratify experimental animals according to their susceptibility to a disease of interest, allowing optimization of experimental designs and pre-clinical trials. It would be valuable to know if veterinary animals from diverse commercial vendors (e.g., pigs) also have different metabolotypes and if so, to understand their potential impact on drug response and clinical trials.

Biomarker discovery is also a very active area of research in metabolomics. Metabolomics is a useful technique to discover putative biomarkers for disease diagnosis, risk prediction, stratification of patient populations and suggest new therapeutic targets. This application makes metabolomics a key ally for driving decisions in pharmaceutical R&D.

Pharmacometabolomics and individualized medicine
Application of metabolomics for the study of drug effects and variation in drug response is known as “pharmacometabolomics”. Pharmacometabolomics reveal biomarkers for drug response phenotypes, providing an effective means to predict variation in a subject’s response to treatment. In a pioneering study with acetaminophen, Clayton and colleagues demonstrated the connection between an individual's pre-dose urinary metabotype, and the metabolic fate acetaminophen. Individuals having high pre-dose urinary levels of p-cresol sulfate had low post-dose urinary ratios of acetaminophen
sulfate to acetaminophen glucuronide. Pharmacometabolomics can also be used to inform pharmacodynamics studies. Among the most remarkable studies in this area, is the correlation between the urine and plasma metabotype and SSRIs treatment outcomes and novel response pathways. More recently, Rivera and col., revealed altered metabolic pathways in plasma, urine and kidney tissue induced by the repeated administration of meloxicam to cats. In addition, results of this study identified several low molecular weight substances that can serve as putative biomarkers for monitoring the administration high doses of anti-inflammatories to cats. This is probably the first pharmacometabolomics study in the field veterinary medicine.

Although pharmacometabolomics is, at the moment, not extensively applied in veterinary medicine there is no doubt that its applications will soon grow and will help to advance veterinary pharmacology and toxicology research. Pharmacometabolomics has the potential to accelerate the discovery of biomarkers and predict the responses of drugs or adverse effects for individuals, thus, prompting to move from population medicine to individualized medicine.

**Metabolomics-artificial intelligence in veterinary pharmacology**

Biomarkers discovery can be benefited markedly from machine learning. Machine learning is a sub-branch of artificial intelligence in which patterns and statistical models are extracted from large data sets to train algorithms to identify patterns and interactions within large data sets (e.g., metabolomics data). These learned patterns can be applied to test data sets of interest to make outcome predictions.

**Ongoing developments for global clinical metabolomics**

One of the factors limiting multicenter and global studies is the stringent requirements for sample collection. There are continuing efforts for developing methods for metabolomics studies based on dried blood or dried urine spots samples. The collection of dried blood or dried urine spots on absorptive materials, followed by ambient temperature delivery by post to storage facilities, would permit low-cost and larger-scale global metabotyping studies.

**Concluding remarks**

Despite potential benefits, adoption of metabolomics in veterinary pharmacology research has been relatively slow with few exceptions. Metabolomics can enrich decision making from discovery through the clinic. The incorporation of metabolomics in veterinary pharmacology and toxicology research can transform our understanding of mechanisms of drug action and molecular basis for variation in drug response accelerating the translation of novel treatments to the clinic.

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Improving the Effectiveness of Tramadol in Dogs Through Understanding CYP Metabolism

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Preventing and managing pain in dogs has become a fundamental part of the quality of veterinary care and it is essential for overall well-being. As veterinarians we have the ethical responsibility to recognize, assess, prevent and treat pain in animals under our care. Untreated pain can decrease the quality of life of our patients, prolong healing and recovery, and increase the risk of possible complications. Our role in pain management is not complete until we implement an analgesic plan.\(^1\) Analgesics commonly used in dogs are the opioids, the non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and analgesic adjuncts such as ketamine, gabapentin, local anesthetics, and the alpha-2 agonists. The choice of drug is going to depend on the underlying cause of pain and the severity and duration\(^2\) of pain. Knowledge of the pharmacology of analgesic drugs in our patients is required to optimize drug selections.\(^3\)

Tramadol is widely used in dogs for the management of mild to moderate pain conditions (post-surgical or chronic) and can be used alone or in combination with other drugs as part of a multimodal approach to treat pain.\(^4,5\) Tramadol has few side effects and possesses a very low potential for abuse, which makes it an appealing choice for the long term treatment of pain. Even though tramadol was recently placed into schedule IV of the Federal controlled substances act, this has not noticeably impacted the prescription of tramadol in dogs by veterinary practitioners.

Even though tramadol is widely used for treatment of pain by veterinary clinicians, this use is highly controversial. Clinical studies evaluating the analgesic effectiveness of tramadol in dogs have produced conflicting results. In some studies tramadol seems to be more effective\(^6-11\), as effective\(^12-17\) or worse\(^11, 18-20\) than concurrently studied drugs.

Tramadol is considered a pro-drug since it is not itself analgesic, but must be metabolized in the liver to produce the active analgesic metabolite, O–desmethyltramadol (M1). The other major metabolite, N-desmethyltramadol (M2) is considered inactive.\(^21, 22\) Tramadol is also stereactive and is normally administered as a racemic mixture of the two stereoisomers (+ and -). (+)-M1 and (-)-M1 are formed from (+)- and (-)-tramadol, respectively. (+)-M1 is ~4-fold more potent than (-)-M1 in terms of µ opioid-receptor affinity and both (+)- and (-)-M1 are 200-fold more potent than (+)- or (-)-tramadol.\(^23, 24, 25\)

Tramadol is metabolized in humans to M1 by cytochrome P450 (CYP) 2D6 and to M2 by CYP2B6.\(^22-26\) CYP2D6 is one of the most highly polymorphic drug metabolizing enzymes with up to 10% of people having genetic deficiency of this enzyme.\(^27\) Importantly, human patients administered tramadol who were also CYP2D6 poor metabolizers showed reduced tramadol analgesic efficacy. Specifically, these patients had higher analgesic consumption and needed rescue medication more often than patients with faster CYP2D6 metabolism.\(^28\) In addition, patients are often co-administered drugs with tramadol that inhibit M1 formation and decrease its analgesic efficacy. Specific inhibitors of the cytochromes responsible for metabolizing tramadol in dogs have been studied (quinidine and quinine inhibit canine CYP2D15)\(^29,30,31\)
and their inhibition profile is similar in humans, but their impact on tramadol metabolism and efficacy in the clinical setting are unknown.

It is expected that factors influencing M1 formation in dogs will also influence the analgesic effectiveness of tramadol in this species. Based on previous pharmacokinetics studies, dogs differ from humans in that M1 formation is considerably lower. Consequently, tramadol would be expected to be a less effective analgesic. Indeed, low although detectable analgesia (reduced pain pressure thresholds) was observed in a pharmacokinetic/pharmacodynamic study of 6 dogs administered 10 mg/kg of tramadol orally. However, these studies also revealed a significant inter-individual variability (5-fold) in M1 formation between dogs.

Based on these findings we proposed that variable efficacy of tramadol in clinical studies in dogs is a consequence of variable M1 formation either from genetic polymorphism or co-administration of drugs that inhibit the CYP enzymes metabolizing tramadol. A group of studies (3) was designed to determine some of the principles behind this statement.

In the first study we initially evaluated species differences in hepatic microsomal metabolism of racemic (±)-tramadol to M1 and M2 to test the hypothesis that M1 formation (relative to M2 formation) is slower in dog liver microsomes compared with cat and human liver microsomes. We then used multiple approaches (recombinant enzymes, chemical and antibody inhibition, and induced hepatic microsomes) to identify the CYPs responsible for metabolizing (+)-tramadol and (-)-tramadol to M1 and M2 in dog liver. We had hypothesized that M1 would be formed by CYP2D15 (the canine ortholog of human CYP2D6), and that M2 would be formed by CYP2B11 and CYP3A12 (the canine orthologs of human CYP2B6 and CYP3A4). The major novel finding of this study is that tramadol is metabolized in dog liver to M1 by CYP2D15, while M2 is formed by multiple enzymes, primarily CYP2B11 and CYP3A12. Multiple observations provide evidence supporting these conclusions, including recombinant enzyme activities, selective inhibition of M1 formation by quinidine, and of M2 formation by chloramphenicol and CYP2B11 antisera, and induction of M2 formation (but not M1 formation) by phenobarbital. Another important finding was the marked species difference in formation of M1 and M2 by dog, human, and cat liver microsomes. Based on published data on tramadol and metabolite plasma concentrations in dogs, we had hypothesized that M1 formation should be lowest with DLMs. Although we did confirm lower M1 formation compared with cats, M1 formation by DLMs was higher than for human liver microsomes. Consequently, additional mechanisms are likely to account for low M1 concentrations in dog plasma. One possibility is competition for substrate availability for O-demethylation to M1 by more rapid N-demethylation to M2. In conclusion, the results of this study suggest that lower circulating concentrations of the tramadol M1 metabolite in dogs compared with humans and cats may be explained by more efficient formation of the tramadol M2 metabolite through a competing pathway.

In the second study, we initially evaluated species differences in the formation of (+)-M5 from (+)-M1 and from (+)-M2 by dog liver microsomes, compared with cat and human liver microsomes. We studied the (+)-enantiomers of the metabolites here since, although tramadol is used clinically as a racemic mixture, there is evidence that (+)-M1 is a more potent μ-opioid agonist than (-)-M1, and we have previously observed somewhat faster formation of (+)-M1 from tramadol compared with (-)-M1 formation. We then used different approaches (canine recombinant enzymes, chemical inhibition and hepatic microsomes from inducer treated dog) to identify the CYPs involved in the formation of (+)-M5 from (+)-M1 and from (+)-M2 in dog liver. Given the structural similarities between (+)-tramadol compared with
(+)-M1 and (+)-M2 as substrates (i.e. they only differ by a methyl group that is away from the demethylation site), we hypothesized that O-demethylation of (+)-M2 would be performed mainly by CYP2D15, while N-demethylation of (+)-M1 would be performed by multiple CYPs including CYP2B, CYP2C and CYP3A isoforms. Finally, we used a competitive transport assay to evaluate whether (+)-tramadol, (+)-M1, (+)-M2, or (+)-M5 are substrates of canine P-glycoprotein, which could limit central nervous system distribution via the blood-brain barrier. The results of this study substantially enhance our understanding of the complex pathways involved in tramadol metabolism in dogs (Fig. 1). Based on recombinant enzyme data, we determined that (+)-M1 is metabolized in dog liver to (+)-M5 primarily by CYP2C21 with lesser contributions from CYP2C41 and CYP2B11.

Figure 1. Primary and secondary CYP-dependent pathways of (+)-tramadol metabolism that have been phenotyped in dog liver.

By comparing these latter results to those we previously generated for (±)-M1 formation from (±)-tramadol, we determined that (±)-M1 formation from (±)-tramadol exceeds (+)-M1 metabolism to (+)-M5 suggesting that this latter reaction may be an important rate-limiting step in determining circulating M1 concentrations in the species tested. Consequently, the higher metabolism of (+)-M1 to (+)-M5 (primarily by CYP2C21) in dogs may explain in part the lower circulating (±)-M1 concentrations in dogs compared with humans and cats. The results of our study indicated that (+)-M5, as well as (+)-M1, (+)-M2, and (+)-tramadol are not substrates for P-glycoprotein. Consequently, the poor brain penetration of (±)-M5 compared with (±)-tramadol and (±)-M1 may be a consequence of the higher polarity of this metabolite rather than efflux by p-glycoprotein from the CNS. Alternatively, (±)-M5 might be a substrate for a different transporter such as ABCG2. In summary, we have determined that (+)-M5 is formed primarily from (+)-M2 by CYP2C21 and to a lesser extent from (+)-M1 by CYP2D15 in dog liver microsomes. More rapid N-demethylation of (+)-M1 by dog liver compared with livers from humans and cats may contribute to the relatively low circulating concentrations of (±)-M1 observed in dogs compared with those other
species. The results of this study have enabled a more complete picture of the major CYP-dependent pathways involved in tramadol metabolism in dog liver.

In the third study, the purpose was to identify a drug that could be co-administered with tramadol to increase plasma M1 concentrations, thereby enhancing analgesic effectiveness. We used a two-step approach. Firstly, we conducted an in vitro screen using DLMs to identify a drug that selectively inhibits M2 formation from tramadol and M5 formation from M1, without inhibiting M1 formation from tramadol. We then conducted a proof-of-principle drug-drug interaction study in healthy dogs to test the hypothesis that the identified drug would increase plasma and urine concentrations of tramadol and M1, while decreasing concentrations of M2 and M5. The in vivo study pharmacokinetic results completely confirmed the predictions we derived from the in vitro model (summarized in Figure 2). Markedly higher plasma and urine tramadol concentrations are consistent with overall increased systemic availability of orally administered tramadol via inhibition of metabolism to M2. In prior work 36, we showed that M2 formation is mediated primarily by CYP2B11 and CYP3A12, which are both highly expressed in tissues that contribute to first-pass extraction of drug after oral administration, including intestinal mucosa and liver 38.

Figure 2. Effect of fluconazole on tramadol metabolism in dogs.

In addition to higher tramadol concentrations, we also observed marked (up to 40-fold) increases in plasma and urine M1 concentrations with fluconazole. This effect is likely a consequence of increased CYP2D15 substrate (i.e. tramadol) availability because of shunting of tramadol metabolism away from the M2 pathway, as well as inhibition of further metabolism of M1 to M5. M1 can also be metabolized by conjugation pathways, including glucuronidation and sulfation 39. In agreement with prior studies 39, 40, our results showed that M1 is primarily found as conjugates in the urine and has extended those findings to include plasma conjugates. Consequently, conjugation is likely an important mechanism for elimination of M1 in dogs, and inhibition of M1 conjugation by fluconazole would be another mechanism to explain increased M1 plasma and urine concentrations. Consequently, future studies are needed to determine whether adding fluconazole can enhance the analgesic efficacy of tramadol in healthy dogs (such as through nociceptive threshold testing) and in clinical patients experiencing pain.

REFERENCES
SESSION 2: Graduate Student Presentations

Oral Presentations

Presented by graduate students who are eligible for the ACVCP/AAVPT Resident/Graduate Student Research Award – abstracts included in poster abstracts listed below.
**Poster Abstracts**

(Alphabetical by first author)

**Canine albumin and orosomucoid (α1-acid glycoprotein) polymorphisms and their impact on drug plasma protein binding**

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**Key words:** pharmacogenomics, plasma protein binding, ORM1, genetic variant

**Background:** Genetic variants of the two main drug-carrier proteins (albumin and orosomucoid) can alter plasma drug binding in humans, however, little is known in dogs. The study objective was to determine the frequency of canine albumin and orosomucoid polymorphisms and evaluate their impact on the extent of drug protein binding in plasma.

**Methods:** Albumin (ALB) and orosomucoid (ORM1) genes were sequenced in 100 dogs to identify common gene variants. Allele frequency of common variants was then determined in a larger population (n=1446; 61 different breeds). Protein binding of drugs that preferentially bind to albumin (D01-4582, celecoxib, meloxicam and mycophenolic acid) or orosomucoid (amitriptyline, lidocaine, indinavir and verapamil) was evaluated independently in plasma from dogs with the two most common variants of albumin (n=12) or orosomucoid (n=12). Plasma drug concentrations were quantified by HPLC-MS/MS.

**Results:** The most common variants were c.1075G>T (p.Ala359Ser) and c.1422A>T (p.Glu474Asp) for albumin, and c.70G>A (p.Ala24Thr) for orosomucoid. Variant allele frequency varied by breed. For most drugs, the extent of plasma protein binding did not significantly differ between ORM1 and ALB genotypes (P>0.05). For meloxicam, plasma from dogs with ALB H1 allele (c.1075GG and c.1422AA) had significant higher free drug fractions (P=0.041) than plasma from dogs with ALB H2 allele (c.1075TT and c.1422TT).

**Conclusion:** ALB and ORM1 polymorphisms occur in dogs, with high allele frequencies in some breeds. The presence of albumin H2 allele seems to influence the extent of plasma protein binding of meloxicam and may need to be considered when evaluating pharmacokinetic data from dogs.
Clinical Efficacy of an Oral Long-Acting Analgesic with a Human Abuse Deterrent in Perioperative Dogs

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Currently pain control in the perioperative setting is difficult due to the need for frequent injections of opioid drugs in dogs. A novel long-acting oral methadone formulation containing the human abuse/misuse deterrent naltrexone was evaluated in 43 dogs undergoing an ovariohysterectomy (OHE).

Dogs were randomly assigned to one of three treatments, 1) methadone 0.5 mg/kg SC q4h (positive control, n=13 dogs), 2) methadone 0.5 mg/kg with fluconazole 2.5 mg/kg and naltrexone 0.125 mg/kg PO q12h (n=15 dogs) and 3) methadone 1 mg/kg with fluconazole 5 mg/kg and naltrexone 0.25 mg/kg PO q12h (n=15 dogs). Injectable methadone was started 0.5-3 hr prior to IV catheter placement and oral methadone was started 14-17 hr prior to IV catheter placement. Acepromazine (0.05 mg/kg SC) was administered to all dogs 0.5 -3hr prior to catheter placement. Propofol was administered through the IV catheter to induce anesthesia which was maintained with isoflurane. A standardized OHE was performed, and dogs were monitored for postoperative pain using the validated modified Glasgow Composite Pain Scale (GCPS).

Intravenous catheters were successfully placed in all dogs. Propofol induction doses and GCPS scores were not significantly different (P > 0.05) between treatments. No dogs required rescue analgesia (all GCPS < 5). The most common adverse effect was perioperative vomiting, which was not significantly different between the treatments.

In conclusion, a novel twice daily oral opioid formulation containing a deterrent to human abuse/misuse was effective in providing preoperative sedation with acepromazine and for effectively controlling postoperative pain in dogs undergoing an OHE.

Keywords: methadone, analgesia, post-operative, ovariohysterectomy, naltrexone
The purpose of this study was to assess in dogs a long-acting oral opioid formulation containing a deterrent to human abuse/misuse. We hypothesized opioid effects would be maintained throughout the dosing for both treatments.

Previous studies have documented the combination of methadone/fluconazole/naltrexone provides at least 12 hours of opioid effect when administered as a single dose to dogs previously administered fluconazole. The speed of onset in dogs not pre-administered fluconazole and the effects of multiple doses have not been determined.

Twelve healthy Beagle dogs were divided into two equal groups using a parallel study design. Group 1 received methadone:fluconazole:naltrexone at 1: 5: 0.25 mg/kg repeated ~12 hours later followed by 0.5: 2.5: 0.125 mg/kg approximately q12h PO for a total of 4 doses. Group 2 received methadone:fluconazole:naltrexone at 1: 5: 0.25 mg/kg followed by 0.5: 2.5: 0.125 mg/kg at approximately 4, 10 and 24 hr for a total of 4 doses.

Rectal temperature (previously correlated to analgesia in dogs) was significantly decreased from baseline in both groups (P < 0.05) throughout the dosing (including after the initial dose) and for 12 hours after the last dose (except 1 time point in Group 1). Baseline and subsequent von Frey measurements were variable in both groups, but significant antinociception occurred in both groups including after the first dose. Mean methadone plasma concentrations exceeded 10 ng/mL throughout the dosing.

In conclusion, significant opioid effects and antinociception occurred in dogs after multiple doses of a long-acting oral opioid formulation containing an abuse deterrent.

Key words (5): methadone, pharmacokinetics, opioid, naltrexone, analgesia
Characterization of CYP450 mediated metabolism of the polymorphic CYP2D6 probe drug codeine in horses

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Cytochrome P450s are an important family of enzymes that metabolize both endogenous substrates as well as many therapeutic drugs and xenobiotics. Knowledge of P450 mediated drug metabolism is vital to establishing safe and effective dosing regimens, especially those involving multiple drugs. Identification and characterization of equine P450s, with respect to their involvement in drug metabolism, is still very much in its infancy. This study aims to build on the limited current knowledge regarding P450 mediated metabolism in horses by describing the metabolism of codeine in vitro.

Codeine, at varying substrate concentrations, was incubated with equine liver microsomes (± UDPGA) and a panel of baculovirus expressed recombinant equine P450s. Parent drug and metabolite concentrations were determined using LC-MS/MS. Incubation of codeine in equine liver microsomes generated norcodeine, morphine, codeine glucuronide, M3G and M6G. In recombinant P450 assays, the newly described CYP2D82 was found to be responsible for catalyzing the reaction of codeine to morphine (Km of 139.6 μM and a Vmax of 2.06 pmol/min/pmol P450). CYP2D82 is 80% homologous to the highly polymorphic CYP2D6 enzyme, which is responsible for biotransformation of codeine to morphine in humans. CYP3A95, which shares 79% sequence homology with human CYP3A4, catalyzed the conversion of codeine to norcodeine (Km of 104.1 μM, Vmax of 2.76 pmol/min/pmol P450). In addition to describing the P450 mediated metabolism of codeine, the current study offers a candidate probe drug that could be used in vivo to study the functional implications of polymorphisms in the CYP2D gene in horses.

Key words: Cytochrome P450, codeine, horse, probe drug, metabolism
Metabolism, pharmacokinetics and selected pharmacodynamic effects of codeine following a single oral administration to horses

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In humans, codeine is a commonly prescribed analgesic that produces its therapeutic effect largely through metabolism to morphine. In some species, analgesic effects of morphine have also been attributed to the M6G metabolite. Although an effective analgesic, administration of morphine to horses produces dose-dependent neuroexcitation at therapeutic doses. To date, there have been no studies describing the metabolism, pharmacokinetics or analgesic efficacy of codeine in horses. Our laboratory has hypothesized that codeine administration will provide effective analgesia with decreased adverse excitatory effects due to slower biotransformation to morphine and production of morphine-6-glucuronide. In the current study, we take the first step in testing this hypothesis by describing the metabolism, including presumed conversion to morphine and M6G, pharmacokinetics and select pharmacodynamic parameters of codeine following a single oral administration to horses. To that end, 12 horses received a dose of 0.6 mg/kg codeine. Blood was collected before administration and at various time points until 120 h post administration. Plasma samples were analyzed for codeine and its metabolites by liquid chromatography-mass spectrometry. The subsequent data was evaluated using a two-compartment population model. Pharmacodynamic data was collected prior to and until 4 h post administration. Codeine was rapidly converted to morphine and both M3G and M6G, with M6G concentrations equivalent to previous reports following high dose morphine administration. Codeine concentrations were below the LOQ for all twelve horses by 18 h. The $C_{\text{max}}$, $T_{\text{max}}$, and elimination $t_{1/2}$ for codeine were 209.8 ng/mL, 0.31 h, and 0.50 h respectively. No significant adverse or excitatory effects were observed.

Key words: horse, metabolism, codeine, pharmacokinetics, pharmacodynamics
Horses efficiently and extensively glucuronidate a number of xenobiotics making uridine diphospho-glucuronosyltransferases (UGTs) an important group of drug metabolizing enzymes for the clearance of drugs in this species. UGTs are membrane bound enzymes that catalyze the conjugation of glucuronic acid onto a diverse set of xenobiotics. The use of recombinant enzymes has allowed researchers to characterize the metabolism of a variety of drugs. The goal of the current study was to clone, express and characterize equine UGTs using morphine as a substrate. Four UGT variants were selected from the NCBI equine genome database based on homology to the human UGT2B7, the well characterized enzyme responsible for metabolizing morphine in humans. cDNA sequences were cloned and resulting protein expressed in a baculovirus expression system. Functionality of the enzymes was assessed using the commonly glucuronidated substrate 4-methylumbelliferone (4-MU). Each recombinant enzyme, non-expressed control cells, equine liver microsomes and commercial human recombinant UGT2B7 were then incubated with morphine and concentrations of metabolite measured using LC-MS/MS. Based on homology to the human UGT2B7, four equine UGT variants were expressed: UGT1A1, UGT2A1, UGT2B31, and UGT2B4. Of these variants, UGT2B31 metabolized morphine into significant amounts of morphine-3 glucuronide and trace amounts of morphine-6 glucuronide. To the authors’ knowledge, this is the first successful expression of functional recombinant equine UGTs. These results warrant further investigation into UGT2B31 as a contributor to morphine metabolism and investigation into potential polymorphisms that may contribute to the variation in morphine metabolism in vivo.

Keywords: horse, UGT, morphine, metabolism
Pharmakokinetics and Pharmacodynamics of oral oxycodone administration in horses

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Oxycodone, a µ-agonist commonly prescribed to alleviate pain in humans, has been detected in plasma and urine samples collected for equine post-race drug testing. The pharmacokinetics and pharmacodynamics of oxycodone (5 mg) administered orally to 12 healthy horses (6TB, 6 SB, 8.6 ±2.7 years) was investigated. Plasma and urine samples were collected for up to 96 hours. Oxycodone concentrations were measured using a validated LC-MS/MS method (lower limit of quantification (LLOQ) = 2.5 pg/mL for both matrices). Video monitoring and ECG recording was performed before and after oxycodone administration to 6 horses, and videos and data were evaluated by equine behavioral and cardiac specialists. Oxycodone plasma concentration versus time data best fit a two compartmental model with a weighting factor of 1/Y² and a lag time based on visual inspection of the residual plots, the Akaike information criterion (AIC), the Schwartz information criterion (SIC) and minimizing the coefficients of variation for the estimated parameters. The absorption and distribution phases were very rapid (K01 τ½ = 0.1 (0-0.3) h; α τ½ = 0.4 (0.1-1.4) h, respectively), and were followed by a slower but still fast elimination phase (β τ½ = 3.3 (0.9-35.1) h). The oxycodone concentration was below the LLOQ in plasma by 10 (8-20) hours and in urine by 72 (12 to >96) hours. No behavioral or ECG abnormalities were observed. Ongoing studies are in progress to determine if exposure to oxycodone in the environment could result in its detection in equine blood and/or urine.

Key Words: Opioid, Equine, Pharmacology, Drug Testing
Feasibility of Hepatic Fine Needle Aspiration as a Minimally Invasive Sampling Method for Gene Expression Quantification of Pharmacogenetic Targets

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Variability in hepatic expression of drug transporters and enzymes can significantly impact pharmacokinetics, drug efficacy, and adverse reactions. Research on gene expression in clinical patients has been limited by the necessity of biopsies, which carries inherent risk and may not uniformly represent underlying pathology without acquiring multiple samples. The utility of minimally invasive sampling techniques, such as fine needle aspiration (FNA), as well as the effect of sampling location on hepatic gene expression has not been evaluated in veterinary medicine. We hypothesized that hepatic gene expression can be accurately measured from FNA samples and will not differ based on sampling method or location. Biopsy and FNA samples were acquired in triplicate from central and peripheral locations of the right and left lateral liver lobes of a healthy liver. Relative expression of ABCC1, GSTT1, and CYP3A12 were measured via reverse-transcriptase, quantitative PCR. The effects of sampling method, lobe, and location on expression were assessed using a 3-way ANOVA. Expression of all targets were detectable in all FNA samples. Sampling method did not affect relative expression of ABCC1 or GSTT1, and CYP3A12 were measured via reverse-transcriptase, quantitative PCR. The effects of sampling method, lobe, and location on expression were assessed using a 3-way ANOVA. Expression of all targets were detectable in all FNA samples. Sampling method did not affect relative expression of ABCC1 or GSTT1, but did impact CYP3A12 expression (p=0.013). Lobe sampled affected ABCC1 expression (p=0.001) and site within lobe affected ABCC1 (p=0.018) and GSTT1 (p=0.025) expression. All differences identified were statistically significant but small. FNA appears to be a feasible technique for minimally invasive evaluation of hepatic gene expression, but results cannot be directly compared to biopsy samples. Sampling location impacts expression of some targets; combination of FNAs from multiple sites may reduce this variation.

Key words: Pharmacogenetics, pharmacogenomics, canine, liver, qPCR
Practical and affordable tick prevention in horses in Grenada, West Indies

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Tick parasitism can cause serious equine welfare and health issues through transmission of pathogens. A previous study revealed that 75% and 7% of Grenadian horses and donkeys were seropositive for *Theileria equi*, and *Babesia caballi* respectively. We hypothesize that commercial cattle ear tags impregnated with abamectin applied around the neck of horses by means of a collar, will result in the distribution of the drug over the body of the horse at levels sufficient to kill and repel ticks. Seven clinically healthy horses were fitted an EquestriSafe® Multipurpose Collar with two XP 820™ Ear Tags (Y-Tex, Cody, WY, USA) of 20% piperonyl butoxide and 8% abamectin attached and assessed at three time points over 20 days. Tick burden was assessed using photographs of each animal to quantify tick numbers. One pinna (left) and the perineum were imaged at day 0, 6 and 20. Five independent individuals assigned percentage coverage of the ticks in those regions. The results of the pooled median tick percentages show a significant difference (p < 0.05) in tick numbers over time from 80% at time 0, to 5% at 20 days of the treated horses. The control horses tick counts did not show this trend (27% at time 0, 20% at 20 days). Further research to assess the concentration of the abamectin in the hair and stratum corneum over the same time period, and to extend the time of the study to ascertain time of efficacy is warranted.
Tetrasodium EDTA: a non-antibiotic antimicrobial lock solution effective against clinically relevant microorganisms from Central Venous Access Devices of Canadian patients.

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Key words: alternative to antibiotics, biofilm, tetrasodium EDTA, antimicrobial stewardship, superbugs

Objective: To test the efficacy of a non-antibiotic antimicrobial catheter lock solution containing 4% tetrasodium EDTA (T-EDTA) to eradicate clinically relevant microbes colonising CVADs of (human) patients.

Methods: Clinical strains, from western and eastern Canada, were isolated from over 300 tips of CVADs of TPN, hemodialysis and oncology patients. Microbiological assays were performed to determine the Minimum Biofilm Eradicating Concentration (MBEC) when exposed to the lock solution. Isolates included Staphylococcus, Enterococcus, Candida, Klebsiella, and Pseudomonas species, as well as methicillin-resistant-Staphylococcus aureus (MRSA) and Vancomycin-resistant-Enterococcus (VRE).

Results: T-EDTA eradicated all microorganisms at minimum bactericidal concentrations far below 4%. For recalcitrant biofilms, which are notoriously more difficult to treat, exposure to 4% T-EDTA resulted in greater than 4 logs killing for each species, and complete killing of MRSA and VRE well within 24 hours.

Conclusions: The rise in antimicrobial resistance has led to a continual search for ways to kill pathogenic microorganisms effectively and reproducibly without the use of antibiotics. These in vitro results highlight the ability of 4% T-EDTA to reduce bacterial burden and biofilms within CVADs. This was supported by its clinical use in Canadian patients over the last 2.5 years where 71% and 100% reductions in catheter related bloodstream infections in adult (p = 0.04) and pediatric (0.002) patients respectively were reported. Furthermore, 4% T-EDTA should also be considered as an anti-biofilm agent for increased antimicrobial performance in recalcitrant wounds. Therefore, T-EDTA represents a promising alternative to antibiotics against bacteria including superbugs in line with Antibiotic Stewardship programs worldwide.

References:
Development and application of interactive physiologically based pharmacokinetic (iPBPK) modeling platform to estimate withdrawal intervals for drugs in food-producing animals across species, ages, administration routes and doses

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Physiologically-based pharmacokinetic (PBPK) modeling is a mechanistic-based approach used to estimate drug residues in tissues and animal by-products and withdrawal intervals for food animals across species, age, sex, administration routes and doses. Traditional PBPK models are difficult to use by individuals with no modeling experience. Therefore, a user-friendly PBPK modeling framework and web-based interface would be beneficial. Penicillin G and flunixin meglumine are commonly used drugs for treating food animals and were among the top five violative drug residues identified by the U.S. National Residue Program from 2010-2018. The objective of this study was to establish a web-based user-friendly framework to develop PBPK models for drugs in food animals using penicillin G and flunixin meglumine as case studies. Traditional PBPK models for penicillin G and flunixin meglumine in market-age swine and cattle were developed and validated based on published data from the FARAD Comparative Pharmacokinetic Database¹⁻². The penicillin G model was extrapolated to dairy cows³ and heavy sows⁴. This traditional penicillin G model was converted to a web-based interactive PBPK (iPBPK) framework using R Shiny application. To test the applicability of this iPBPK framework, a new iPBPK model for flunixin meglumine in market-age swine and cattle was created⁵. These PBPK models can be used to predict withdrawal intervals of penicillin G and flunixin meglumine after extralabel administration to swine and cattle across production classes, administration routes and doses in real time. The iPBPK framework presents proof-of-concept for developing web-based iPBPK interfaces for withdrawal interval estimations and food safety assessment.


**Key words:** Food Animal Residue Avoidance Databank (FARAD); Food safety; Physiologically based pharmacokinetic (PBPK) modeling; Tissue residue; Withdrawal period
PHARMACOKINETICS AND IMPACT OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ON BIOMARKERS OF PAIN AND INFLAMMATION AT PIGLET CASTRATION

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Keywords: Piglet, Castration/Processing, Pain, NSAID, Pharmacokinetics

Piglet processing (including castration/tail-docking) is routinely performed in the US without analgesia. Pain medications, predominately NSAIDs, are used in the EU/Canada to decrease pain associated with processing and improve piglet welfare. The efficacy and required dose are unknown. This study assessed efficacy and pharmacokinetics of three NSAIDs (meloxicam, flunixin and ketoprofen) in piglets undergoing routine processing. Five-day-old male piglets (8/group) received one of 5 randomized treatments; intramuscular saline (SAL CAST), meloxicam (MLX; 0.4mg/kg), flunixin (FLU; 2.2mg/kg), ketoprofen (KETO; 3mg/kg) or sham (SAL SHAM; saline injection, no processing). Two hours post-dose, piglets underwent processing. Drug concentrations were quantified in plasma and interstitial fluid (ISF). Cortisol/prostaglandin E2 (PGE2) were quantified in plasma/ISF, respectively. Plasma Tmax and T1/2 of meloxicam, flunixin and (S)-ketoprofen were 1.21, 0.85 and 0.59h and 4.39, 7.69 and 3.50h, respectively. ISF Tmax were 2.81 and 3.64h for meloxicam and flunixin. SAL CAST cortisol was significantly (p=0.0031) higher than SAL SHAM at processing (2h post-dose), suggesting that the procedure, rather than handling, greatly increased cortisol and is indicative of pain/stress. Cortisol was significantly (p=0.0488) lower in FLU piglets compared to SAL CAST at processing. While no significant differences were shown between treatments for PGE2 in ISF, all NSAIDs decreased PGE2 compared to SAL CAST, and only flunixin maintained inhibition beyond 24h post-dose. Results confirm processing is painful and NSAIDs reduce cortisol/PGE2 post-processing. Flunixin better controls pain in piglets compared to other NSAIDs, and administration prior to processing would improve piglet health and welfare in the US.
Clinical Interactions between Oral Fluconazole and Intravenous Ketamine & Midazolam

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Fluconazole is a commonly prescribed antifungal medication with long treatment periods, often months, for the treatment of systemic fungal disease. Recent studies have shown drug metabolism interactions between fluconazole and perioperative drugs in dogs. This study aimed to evaluate drug interactions between fluconazole and the common anesthesia induction agents intravenous ketamine/midazolam.

Twelve Beagle dogs (eight male, four female) were included. Six dogs received ketamine 7 mg/kg IV, midazolam 0.25 mg/kg IV (KM) and 6 dogs were randomly selected to also receive fluconazole 5 mg/kg PO 24 and 12 hours prior to ketamine/midazolam (KMF). Dogs were monitored for temperature, pulse and respiratory rates. A sedation score (0-4) was assigned by blinded investigators, and length of time was recorded to sternal and standing.

The time to sternal (mean KMF 32.3 versus KM 24.6 minutes) was not different between the groups, but the dogs treated with fluconazole had significantly longer (P=0.002) time to standing (KMF 73 minutes vs. KM 36 minutes) and duration of elevated heart rates compared to baseline (KMF 110 minutes versus KM 25 minutes). One KMF dog had hyperthermia (peak 105.1°F) which resolved spontaneously.

While there is an interaction between oral fluconazole and intravenous ketamine/midazolam, the effects appear minor. The time to standing was approximately doubled, but 72 minutes is a clinically acceptable period of time. Fluconazole had a significant effect on duration of elevated heart rate consistent with prolonged ketamine effects. Based on these data, ketamine/midazolam is not contraindicated in dogs treated with fluconazole.

Keywords: Fluconazole; Ketamine; Midazolam; Sedation; Drug interaction
Pharmacokinetics of oral and intravenous administration of trimethoprim-sulfamethoxazole in Rhode Island Red chickens (*Gallus gallus domesticus*)

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Trimethoprim-sulfamethoxazole (TMP-SMZ), is a broad-spectrum antibiotic. While TMP-SMZ is not FDA approved for use in laying hens in the United States, its use is also not prohibited by 21CFR§530.41. Fifteen 3-year old Rhode Island Red hens were deemed healthy based on a complete physical exam, plasma biochemistries, and packed cell volumes. A single dose crossover study was performed using the five hens. Initially, 5 birds each received TMP-SMZ (Aurobindo Pharma USA Inc., Dayton, NY) as a single intravenous dose of 96 mg/kg (16mg/kg trimethoprim and 80mg/kg sulfamethoxazole). Following a 14-day washout period, the same dose was administered orally to the same birds. Plasma SMZ-TMP concentrations were measured by Ultra-High-Pressure Liquid Chromatography-Mass Spectrometry (UPLC-MS) and pharmacokinetic analysis of both IV and oral data was best fit to a non-compartmental model. After PO administration, trimethoprim had a mean $C_{max}$, $T_{max}$, and AUC of 2.1 μg/ml, 3.6 hr, and 43.82 hr* μg/ml, respectively, as well as a terminal half-life of 13.73 hr. PO sulfamethoxazole had a mean $C_{max}$, $T_{max}$, and AUC of 67.06 μg/ml, 5 hr, and 1337.61, respectively, as well as a terminal half-life of 6.62 hr. The bioavailability of TMP was 89.5% and 54.6% for SMZ.

Keywords: trimethoprim-sulfamethoxazole, Rhode Island Red chicken, backyard chicken, pharmacokinetics

References:
TREATMENT OF OBSTRUCTED SUBCUTANEOUS URETERAL BYPASS DEVICES WITH TETRASODIUM ETHYLENEDIAMINETETRAACETIC ACID SOLUTION (tEDTA) IN CATS

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Keywords: Cats, ureteral obstruction, sub-cutaneous ureteral bypass, tetrasodium ethylenediaminetetraacetic acid

Objective: Describe the use of 4% tetrasodium ethylenediaminetetraacetic acid (tEDTA) solution in cats with mineral obstruction of their SUB device.

Study Design: Retrospective case series.

Animals: Six obstructed SUB devices in 6 cats.

Methods: Cats had placement of SUB devices for benign ureteral obstruction. Tetrasodium EDTA infusion was considered if partial or complete intraluminal obstruction of the SUB device was suspected. Obstruction was confirmed based on the results of ultrasound-guided flush. The SUB device was drained and 0.5 to 3 mL of 4% tEDTA was infused in the system. Following initiation of tEDTA infusions, relief of obstruction was based on normalization of subsequent SUB flushes.

Results: Six cats were included in the study. Five cats had complete SUB device obstruction and one cat had partial obstruction. Improved SUB flushing was noted after a median of 7 infusions (range: 1-10 infusions) and complete relief of the obstruction was noted after a median of 8 infusions (range: 2-14 infusions). Patency could not be achieved in one cat with complete obstruction despite 15 infusions over a 34 day-period. Three cats experienced mild self-limiting lower urinary tract signs following tEDTA infusion.

Conclusions: Mineral obstruction of SUB devices may be successfully treated with tEDTA infusions. Relief of obstruction was achieved in the majority of patients thus avoiding invasive surgical tube replacement.
Identifying the source of arsenic residues following parenteral versus oral dosing in horses

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Australian racing laboratories routinely screen equine urine using ICP-MS for arsenic, to enforce a urinary arsenic threshold of 300ng/mL. Trainers of horses whose samples exceed the threshold have claimed that the elevated urinary arsenic concentration results from consumption of arsenic-chromium treated fencing timbers. Previously we have shown that feeding horses 100g sawdust per 500 kg bodyweight or a single IV dose of 1mg/kg sodium arsanilate (Jurocyli; CEVA) to individual horses resulted in urinary arsenic concentrations above the threshold. Urinary chromium could be detected but differentiation between the dose’s source was not possible (AEC 1513794.2). We now report that mane-hair samples were also collected by plucking at 4 weeks post-dose and analysed using laser-ablation ICP-MS. Hair from a control horse and from one horse of each treatment group were analysed twice. First, LA-ICP-MS was directed along the cortex of the hairs and second, the hairs were split and the laser was directed along the core of the hairs. In the first analysis, a strong signal for As (m/z 75) was detected in hair from the treated horses, at points along the hair shaft corresponding to dose-timing with hair growth rate of approximately 2cm/mo. In the second analysis, a strong signal for Cr (m/z 52) was detected only in the orally treated horse’s hair at the relevant hair shaft length. We conclude that LA-ICP-MS using hair samples is a useful tool for retrospective confirmation of crib-biting of As-treated timber which can result in urinary arsenic detection.

Advantages of a likelihood approach for regression analysis of pharmacokinetic and pharmacodynamic data.

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Though non-linear mixed-effects modelling (NLME) is now routine in many applications in pharmacokinetics and pharmacodynamics, regression (‘two-stage’) analysis remains popular, including in veterinary science. Regression analyses in quantitative pharmacology have typically been conducted using variations of the weighted sum-of-squares (WSS) approach, which have intuitive meaning in the context of parameter estimation. In many other applications, WSS is often substituted by maximum likelihood estimation (MLE), a probabilistic interpretation of model optimization. MLE underlies many of the familiar estimation procedures in NLME, but has rarely been described for regression analysis. It is proposed here that the capabilities of regression analysis of pharmacokinetic and/or pharmacodynamic data may be expanded by the use of MLE. Working with the likelihood function, as a probabilistic measure of model goodness-of-fit, facilitates extensions to the typical practice of regression analysis in quantitative pharmacology. Whereas censored observations, for example those below an assay performance limit, must be removed or substituted by an arbitrary value in WSS, they may be handled as partially observed in MLE. For multivariate models (i.e. with multiple simultaneous response variables), WSS approaches present difficulty regarding the selection of relative weights for the responses, especially where they are observed on different scales (e.g. in PK-PD or physiologically-based models), but in MLE this may be handled by specifying an error model for each response. Finally, use of the likelihood facilitates uncertainty analysis at the subject-level, using recently developed methods involving the profile likelihood function. These characteristics are demonstrated in a case study.
A physiologically based pharmacokinetic model of doxycycline for predicting tissue residues and withdrawal intervals in grass carp (*Ctenopharyngodon idellus*)

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Physiologically based pharmacokinetic (PBPK) models are an efficient tool in the prediction of tissue residues and withdrawal intervals of drugs in domestic food-producing animals and fish. The objectives of this study were to determine the tissue residue depletion kinetics and to establish a PBPK model of doxycycline in grass carp (*Ctenopharyngodon idellus*). Residue depletion kinetic data for doxycycline were collected at predetermined time points (0.25, 0.5, 1, 3, 5, 7, 14, 21, 28, 35, 42, 49 and 56 days) in plasma, liver, kidney, gill, and muscle+skin of grass carp after repeated oral administrations at 20 mg/kg for 3 days. These data were used to calculate withdrawal intervals using FDA’s tolerance limit method. Based on these data, a seven-compartment PBPK model including plasma, liver, kidney, gill, muscle+skin, richly and slowly perfused tissues was developed. Physiological parameter values such as cardiac output and tissue weights were measured experimentally. Partition coefficients were calculated using area-under-the-concentration method. Other parameters were from the literature or estimated by fitting to the collected data. Preliminary results showed that this PBPK model properly captured the observed kinetic profiles of doxycycline in the plasma and edible tissues in grass carp after multiple oral administrations. Model optimization and evaluation are ongoing. This model provides a useful tool to predict tissue residues and withdrawal intervals of doxycycline in grass carp to aid food safety assessment, and also serves as a foundation for extrapolation to other fish species.

**Key word**: physiologically based pharmacokinetic (PBPK) model, grass carp, drug residue, food safety, withdrawal period
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SESSION 3: Antimicrobials and Diagnostic Laboratories

ANTIMICROBIAL SUSCEPTIBILITY TESTING: BREAKPOINT SETTING HISTORY AND PERSPECTIVE

The Clinical and Laboratory Standards Institute (CLSI) Veterinary Antimicrobial Susceptibility Testing (VAST) subcommittee has been active since 1993. Before VAST started developing veterinary-specific breakpoints, all susceptibility testing for bacteria isolated from animals used the human standards (M100). Testing standards for bacteria isolated from animals were needed to provide the best guidance for drug selection, interpretation, and monitoring programs. The latest edition of the published standard is the 5th edition (CLSI VET01, 2018). The accompanying document published as a supplement (VET08) has tables with antimicrobial agents recommended for testing, interpretive categories and breakpoints, and quality control (QC) ranges. The most recent version is in its 4th edition (CLSI VET08, 2018). The VAST subcommittee has developed breakpoints for 186 drug-bug combinations. Since 2015, there have been 41 new breakpoints with 34 of these developed by the Generic Drug Working Group (described below).

The mission of CLSI-VAST is to develop and promote performance standards, breakpoints, and interpretive categories for in vitro antimicrobial susceptibility testing of bacteria isolated from animals. Participation in VAST is entirely voluntary. Not all laboratories use CLSI standards. However, it is the only global organization with published susceptibility testing standards for animals. (The European veterinary subcommittee of EUCAST, VetCAST has not published any standards for interpretation at this time.) If a laboratory does not adhere to a public standard such as CLSI, susceptibility testing interpretation may be inconsistent from laboratory to laboratory and between countries.

All members of CLSI-VAST are volunteers. The committee is composed of representatives from industry, microbiology laboratories, device manufacturers (susceptibility testing companies), government (regulatory), and professions (academia). CLSI uses a consensus-driven process and open meetings to develop standards for testing. The committee is continuously evaluating existing interpretive categories and breakpoints for refinement and revision.

How are Standards Developed?

The most important information for the clinician to guide treatment is the report that informs them which drugs have an “S” and which ones have an “R”. What goes into this interpretation? The paper by Turnidge and Paterson (2007) describes the process of setting breakpoints. The CLSI-VAST uses a published guideline (VET02) to develop their standards and establish breakpoints. Sponsors submitting breakpoints to CLSI must submit data to support a proposed breakpoint. The data includes pharmacokinetic data in the target species, MIC distributions for the pathogens targeted, clinical data from the drug used under field conditions at the approved dose, and pharmacokinetic-pharmacodynamic (PK-PD) analysis, using Monte Carlo Simulations (Ambrose, 2006) to show that, at the approved dose, the drug attains PK-PD targets for the labeled pathogen. For older drugs that do not have active sponsors (referred to as “generic drugs” in the guidelines), or human-labeled antimicrobial agents used frequently in animals the Generic Drug Working Group has developed breakpoints. For
these drugs, the committee will consider less rigorous detail. For example, results from clinical use of the agent under controlled field conditions may not be available.

**Are These Standards, or Guidelines?**
The CLSI is a consensus-driven process and after approval by the subcommittee the standards become public documents. The consensus process involves the development and public open review of documents, revision of documents in response to discussion, and, finally, the acceptance of a document as a consensus standard or guideline. The CLSI documents used for culture and susceptibility testing should be regarded as a public standard, not a guideline.

A Standard is a document developed through the consensus process that clearly identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. A Standard may, in addition, contain discretionary elements, which are clearly identified. A **Guideline** is a document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

**Interpretation of Susceptibility Tests**

Resistance and susceptibility are determined by comparing the organism's MIC (or zone of inhibition) to the drug's breakpoint. After a laboratory determines an MIC, it will use the CLSI “SIR” classification for breakpoints (S, susceptible; I, intermediate, or R, resistant). CLSI refers to these as Interpretive Categories. The recently developed report from CLSI (VET09, 2019), *Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings*, is now available to help laboratories and clinicians with the interpretation. This report, published in July, 2019 was written to provide veterinarians with the information needed to successfully acquire and interpret antimicrobial susceptibility test results. It promotes common understanding between the veterinarian and the veterinary microbiology laboratory by providing example culture and susceptibility reports and animal species-specific guidance on applying breakpoints to interpret susceptibility test results.

In practice, if the MIC for the bacterial isolate falls in the susceptible category, there is a greater likelihood of successful treatment (cure) than if the isolate were classified as resistant. It does not assure success; drug failure is still possible owing to other drug or patient factors (for example, immune status, immaturity, or severe illness that compromises the action of antibacterial drugs), and interactions. If the MIC is in the resistant category, bacteriologic failure is more likely because of specific resistance mechanisms or inadequate drug concentrations in the patient. However, a patient with a competent immune system may sometimes eradicate an infection even when the isolate is resistant to the drug in the MIC test.

The intermediate category is intended as a buffer zone between susceptible and resistant strains. This category reflects the possibility of error when an isolate has an MIC that borders between susceptible and resistant. If the MIC value is in the intermediate category, therapy with this drug at the usual standard dosage is discouraged because there is a good likelihood that drug concentrations may be inadequate for a cure. However, successful therapy is possible when the agent concentrates at certain sites – in urine, or as the result of topical therapy, for example – or at doses higher than the minimum effective dose listed on the label. Prescribing guidelines for some antimicrobials allow for an increase in dose when susceptibility testing identifies a bacterial isolate in the intermediate interpretive category.
For example, some fluoroquinolone antimicrobials have been approved with a dose range that allows increases in doses when susceptibility testing identifies an organism in the Intermediate range of susceptibility. In these cases, higher drug concentrations make a cure possible if the clinician is able to safely increase the dose above the minimum labeled dose. (For example, in the case of enrofloxacin for dogs, this would be equivalent to a dose of 10 to 20 mg/kg/day, rather than the minimum dose of 5 mg/kg/day.)

MIC data should not be used in isolation. By coupling the MIC from a laboratory report with CLSI interpretive categories and other important information such as the virulence of the bacteria and the pharmacology of the antibiotics being considered, the clinician can make a more informed selection of an antibacterial drug.

**Does the susceptibility test provide tissue-specific interpretation?**

The susceptibility interpretation is based on plasma/serum concentrations. No tissue-specific interpretation can be provided that accounts for differences in drug distribution among tissues (exception for urine isolates described below). In most instances, the clinician should not be concerned with the question of whether or not there are tissue-specific susceptibility interpretations. Antibiotic unbound drug concentrations in the serum or plasma approximate the drug concentration in the extracellular space (interstitial fluid) for most tissues. This is because there is no barrier that impedes drug diffusion from the vascular compartment to extracellular tissue fluid. The concept of “good penetration” and “poor penetration” when comparing antibiotics is overemphasized by many clinicians. Pores (fenestrations) or microchannels in the endothelium of capillaries are large enough to allow drug molecules to pass through unless the drug is restricted by protein binding in the blood. Tissues lacking pores or channels may inhibit penetration of some drugs (discussed below).

If adequate drug concentrations can be achieved in plasma, there are no barriers in the tissue will prevent drug diffusion to the site of infection as long as the tissue has an adequate blood supply. Therefore, clinicians should be concerned when treating tissues that have poor or impaired blood supply. Drug diffusion into an abscess or granulation tissue is sometimes a problem because in these conditions, the site of infection may lack an adequate blood supply and drug penetration relies on simple diffusion. In an abscess, there may not be a physical barrier to diffusion, but low drug concentrations can occur because of poor blood perfusion and drug concentrations are slow to accumulate.

For some tissues, a lipid membrane (such as tight junctions on capillaries) can present a barrier to drug diffusion. In these instances, a drug must be sufficiently lipid-soluble, or be actively carried across the membrane in order to reach effective concentrations in tissues. These tissues include: the central nervous system, eye, and prostate. There also is a barrier between plasma and bronchial epithelium (blood : bronchus barrier). This limits drug concentrations of some drugs in the bronchial secretions and epithelial fluid of the airways. However, this barrier may be compromised during infection (pneumonia).

**Exception for Urine Isolates.** Even though many antibiotics concentrate in the urine – which is beneficial for treating a urinary tract infection – the susceptibility testing interpretive categories are based on achieving adequate concentrations in the blood. For drugs excreted in the urine in an active form, CLSI allows exceptions for interpretation of some drugs. In the current veterinary standards (CLSI, VET08, 2018), there are separate clinical breakpoints for urine isolates of the Enterobacteriaceae for some β-
lactam antibiotics. The interpretation for amoxicillin, amoxicillin-clavulanate, first-generation cephalosporins, and cefovecin (Convenia), allow for different interpretation because of the high concentrations these drugs achieve in urine. This interpretation assumes that (1) the infection is confined to the lower urinary tract, (2) other structures are not infected such as the prostate or kidney, and (3) the animal can sufficiently concentrate the urine (eg, no evidence of chronic kidney disease, or medications that may dilute the urine).

The “S” breakpoint for systemic infections (eg, skin and soft-tissue) in small animals for ampicillin/amoxicillin and amoxicillin-clavulanate is ≤ 0.25 µg/mL. But when the isolate is identified from the lower urinary tract, a higher “S” breakpoint of ≤ 8 µg/mL can be used. The higher breakpoint was derived from studies showing that when these drugs are administered orally to dogs, concentrations in urine are many times higher than systemic concentrations (eg, > 300 µg/mL). The high urine concentrations produced clinical effectiveness for treating infections caused by Staphylococcus spp, and Enterobacteriaceae that would otherwise have been categorized as resistant according to the conventional (non-UTI) interpretive categories.

Higher breakpoints are also established for first-generation cephalosporins and cefovecin when interpreting urine isolate susceptibility. The cefazolin and cephalaxin breakpoint of ≤ 16 µg/mL (“S”) can be used to predict the susceptibility of cefazolin and the oral first-generation cephalosporins instead of ≤ 2 µg/mL used for other tissues. A breakpoint of for cefovecin of ≤ 2 µg/mL (“S”) can be used for urinary tract isolates from dogs instead of ≤ 0.5 µg/mL which applies to skin infections. Cefpodoxime, an approved oral cephalosporin for dogs, may be tested individually because some isolates may be susceptible to this agent while testing resistant to cefazolin or cephalaxin.

A higher urinary breakpoint for cephalosporins offers an alternative for treatment instead of relying on fluoroquinolones and carbapenems. Higher breakpoints for first-generation cephalosporins and cefovecin are justified because of the studies that show high concentrations in urine after standard dosages, and efficacy for treating uncomplicated infections at these dosages. A higher breakpoint of (“S”) of ≤ 16 µg/mL for first-generation cephalosporins will produce a “susceptible” test result for cephalaxin for over 90% of E. coli and Proteus mirabilis isolates from dogs (Moyaert, et al, 2016). On the other hand, using a cephalaxin breakpoint of ≤ 2 µg/mL would cause all of the Enterobacteriaceae isolates to be classified as “resistant” and would drive more use of fluoroquinolones, carbapenems and other highly active agents for treating bacteria of the Enterobacteriaceae.

For complicated, infections (patients with other co-existing disease) one shouldn’t assume that concentrations in urine – even when they are high due to concentration by the nephrons – are sufficient to eradicate infections of the urinary tract. Infections may involve the deeper layers of the mucosa, the renal tissue, or the prostate tissue. In these instances, it is the tissue concentration – which is correlated to the plasma concentration – that will be predictive of a bacteriologic cure (Frimodt-Møller, 2002).

**Summary**

This description of the history of CLSI-VAST and its current activities, illustrates the cooperation among its members to provide the most accurate susceptibility testing information for bacteria isolated from animals. As the work of the committee moves forward, new breakpoints, interpretation guidelines, and educational materials will continue to help clinicians and laboratories.
References


DIAGNOSTIC LABS, BREAKPOINTS AND ANTIMICROBIAL RESISTANCE

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Background

Veterinarians depend on laboratory medicine every day. A clinician’s medical decision are based on the use of results of different laboratory tests; therefore, diagnostic testing is an integral part of clinical medicine. Diverse factors can be associated with the inadequate use of the clinical diagnostic laboratory (CDL) in veterinary medicine. While some of these factors are strictly related to situations that are not controlled by the clinician, such as cost of testing, a limited testing availability, and lack of harmonization among the different methods used, the other factors are influenced by the clinician, such as their lack of knowledge when selecting the best diagnostic test, poor sample collection, and inappropriate interpretation of the results.

The process of laboratory testing comprises three phases, pre-analytical, analytical and post-analytical [1, 2]. These phases are crucial for performing diagnostic testing and must be considered by clinical microbiology laboratories (CML) the veterinarian when requesting a test. The optimal use of CDLs is based on the close relationship between the clinician and the diagnostic laboratory. Although molecular diagnostics play a very important role in the specialty of clinical diagnostics in the new era, “culture and susceptibility” remains as one of the most common requests received by the CMLs. Since antimicrobials were discovered, different antimicrobial susceptibility methods have been employed. CMLs implement the most convenient method according to the number of requested tests, sample origin (animal species) and cost of testing. To ensure that the selected method(s) is properly employed and the results are accurate, CMLs should implement a quality assurance program [3, 4]. Therefore, when performing antimicrobial susceptibility testing, CMLs, researchers and the pharmaceutical industry must adhere to the guidance and standards of the Clinical and Laboratory Standard Institute (CLSI).

The emergence of antimicrobial resistance (AMR) in the bacteria that have clinical importance in human and veterinary medicine has been considered as a worldwide emergency. Under the concept of One Health, international and national organizations have embraced a fight against AMR [5]. Passive and active surveillances are some of the most important components of these worldwide initiatives. CMLs provide antimicrobial susceptibility results as part of the passive surveillance data collection. Hence, the quality of AST results is very important not only for testing clinical cases but also for providing data to national and international AMR databases. Moreover, understanding how the phases of clinical diagnosis are affected by the decisions made by not only the diagnostician, but also by the clinicians would be helpful to excel AST use and interpretation. This document describes the phases that encompass the diagnostic testing cycle and clarifies how mistakes in these phases can generate errors in the process and interpretation of AST. In addition, the author presents how laboratories use the CLSI standards and the challenges faced by veterinary laboratories in properly implementing AST. The goal of this manuscript is to ensure that veterinary CDLs, pharmacologists, and clinical microbiologist understand the importance of AST for medical decision-making and as a tool in the urgent battle against AMR.
Phases of clinical laboratory testing in Clinical Microbiology Laboratories

The pre-analytical phase of clinical laboratory testing comprises the time required and all processes involved for the preparation of a patient for a diagnostic investigation until to the moment the investigation is made. The analytical phase comprises the time and all the processes of a diagnostic investigation. This phase starts when the samples are received by the laboratory. The post-analytical phase comprises the time and all processes for reporting the results of the diagnostic investigation to the person who then undertakes the medical management of the patient [1, 6]. The laboratory personnel controls and is fully responsible for the analytical phase; nevertheless, other personnel such as clinicians and veterinary technicians along with the CDL are accountable for the pre and post-analytical phases.

The quality of the samples collected when requesting culture and susceptibility is crucial for obtaining accurate laboratory results, and it is even more important if antimicrobials are part of the medical treatment of the patient. Specimen collection is one of the most important elements of the pre-analytical phase. The CDL is responsible for providing guidance of the ideal sample type(s) and recommending the best transport media for different clinical and herd situations. The veterinarian or veterinary technician should follow these directions or contact the laboratory for any queries. The clinician is also responsible for preparing the patient prior to sample collection as well as for requesting the most appropriate tests, providing a relevant clinical history to the laboratory and ensuring that sample will be delivered to the testing facility in a timely manner. Failure of the laboratories and/or clinicians during this phase can results in incorrect test selection, false negative results, failure to identify the most probable pathogen, and reporting other microorganisms that may represent normal microbiota or contaminants.

The analytical phase includes the reception of samples and testing, and the CDL is accountable for this phase. The laboratory should be accountable for having a standardized testing methodology, trained personnel, and most importantly, own a quality assurance system program. Ideally, CDLs should ideally attempt to get accreditation or at least, for small testing facilities, mimic laboratories that are fully accredited. The “small size or capability” of the laboratory should not justify the lack of an appropriate quality program.

During the post-analytic phase, the laboratory and submitter of the sample should have a very close relationship. Reporting the testing results in a manner that can be properly interpreted by the veterinarian is the laboratory’s responsibility; however, the clinician should have the knowledge and experience to apply these results to improve the patient’s medical condition or the herd situation. Diagnostic reports are valuable only when the information can be used for patient management. It is therefore an obligation of the diagnostic service to provide the results to the clinician in a timely manner so that the results can be interpreted together with the clinical findings for the patient [1].
Culture and susceptibility are some of the most common requests received by CMLs. The accuracy or errors in any of the abovementioned phases above have an impact on the final therapeutic decisions. Some of the mistakes that can occur during the pre-analytical phase include ordering the incorrect test (aerobic vs. fungal culture), inappropriate sample collection (nasal swab instead of transtracheal lavage), and using the wrong transport media (tissue in a container without media), among many others. These pre-analytical phase mistakes affect the analytical phase; for instance, the CML will have a “no growth culture” or it may identify a pathogen that represents “normal microbiota or a contaminant”. In contrast, if no mistakes occur during the pre-analytical phase, but the laboratory does not perform accurate tests (owing to lack of trained personnel, quality system, and standardized techniques), the analytical phase has a high chance of having errors. The CML should have trained personnel, such as a clinical veterinary microbiologist supervising when reporting results, and the clinicians should be capable of making a correct interpretation (knowledge); these two elements are essential to avoid mistakes during the post-analytical phase.

As indicated by Wians, clinicians and laboratorians should recognize that laboratory data, although potentially extremely useful in diagnostic decision-making, should be used as an aid and adjunct to the constellation of findings (e.g., patient history, and physical exam, etc.) relevant to the patient. Laboratory data is never a substitute for a good physical exam and patient history (clinicians should treat the patient, and not the laboratory results). [2, 6] Moreover, veterinarians should be aware of the phases of clinical laboratory testing and the possibilities of errors while using the laboratory; as a result, mistakes will be minimized, the selection of the laboratory will improve, and clinician will be able to provide the best care for their patients.

**AST and breakpoints in the era of AMR**

The threat of AMR to the One-Health concept has been targeted by several national and international organizations. The World Health Organization (WHO) has listed AMR as one of the top ten threats to global health in 2019 [7]. In 2017, as a collaborative effort to battle AMR, the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization for Animal Health and WHO included in the Tripartite Collaboration Plan the urgency of fighting AMR as a priority of the tripartite commitment [8]. The study by White and Hughes provides a detailed description of the national and international initiatives that have already been undertaking and are currently underway in the worldwide battle against AMR [5].

CMLs play a crucial role when performing AST because the quality of the results has a critical and direct impact when treating a patient. The use of empirical antimicrobials has been linked to the development of AMR. Therefore, clinicians are encouraged to make antimicrobial selection decision-making less subjective by using antibiograms or, even better, by using the results from AST. Moreover, tracking the resistance patterns exhibited by different bacterial species is an important component in the fight against AMR. Therefore, the importance of generating accurate results when performing AST cannot be sufficiently emphasized; it is important not only when handling a patient or herd but also for epidemiological purposes. Consequently, one of the focus areas of the FAO action plan on AMR 2016-2020 is to monitor AMR by developing laboratory capability [9].
The two most common methods used worldwide by CMLs when performing AST are those of the CLSI and the European Committee for Antimicrobial Susceptibility Testing (EUCAST). In the United States and many regions outside Europe, the CLSI standards are more commonly followed [10]. The choice of AST methodology is gaining increased importance owing to the growing international focus on AMR, with both systems recommended in the WHO’s Global Antimicrobial Resistance Surveillance System (GLASS) [11].

The methods used by CMLs, research laboratories and the pharmaceutical industry to perform AST include the disk diffusion test (Kirby-Bauer), broth dilution tests (macro and micro dilution), agar dilution test, and Epsilon test (E-test). Similarly, many laboratories use commercially available platforms; however, these platforms implement CLSI standards when designing their systems. The author of this document, recommends the use of the CLSI standards because CLSI is the only organization in the world that provides testing methods and interpretive categories for both animal and human pathogens. The CLSI standards have been used for many decades, are continually updated, are aligned with the International Standards Organizations, and have been integrated into many AMR surveillance programs, CDLs, and regulatory guidelines; therefore, they offer a “one method” approach that will enable global harmonization [12]. The studies by Jorgensen (2009) and Teale (2019) [13, 14] and also, the most recent CLSI veterinary standard [3, 4] provide an overview of the AST methods.

The process of AST and subsequent data interpretation is complex; therefore, CMLs and clinical providers are encouraged to rigorously follow the CLSI guidelines. Further, the understanding the meaning and application of “breakpoints” is critical [15]. The CLSI defines breakpoints as the minimal inhibitory concentration (MIC) or zone diameter value used to categorize an organism as susceptible, intermediate, nonsusceptible, or resistant [3]. Because the CLSI is the only organization establishing breakpoints and interpretative categories specific to veterinary medicine, their standards should be incorporated by veterinary CDLs. However, it is important to understand that not all the scenarios that can be presented in a veterinary CDL will be covered by the CLSI. The standards for performing the methods are very well defined, but the lack of breakpoints for particular pathogens in a specific animal species is a limitation of these standards (e.g., when testing *Bordetella bronchiseptica* isolated from a horse with pneumonia). The laboratory also should be aware that although the goal of the CLSI's Subcommittee on Veterinary Antimicrobial Susceptibility Test is to establish veterinary-specific breakpoints to decrease reliance on human medical breakpoints, human medical breakpoints are still listed in the most current standards used for AST interpretation [4]. Moreover, some issues affecting the accuracy and proper interpretation of AST results still exist, including the lack of harmonization of methods and breakpoints specific to veterinary medicine as well as the inadequate training of laboratory technicians, supervisors and laboratory users [15-17].

CMLs should be led by a very well-trained clinical veterinary microbiologists as they are responsible for ensuring accurate performance of AST and minimizing the mistakes in each of the phases of the clinical diagnostic process. In addition, the clinical microbiologists should join efforts with veterinary pharmacologists and other professionals with the goal of establishing better guidelines and breakpoints specific to veterinary medicine. The microbiologist may also work together with veterinarian epidemiologists in improving the surveillance methods for AMR. Veterinary pharmacologists and microbiologists should be aware of the new molecular technologies are being proposed as an alternative
for AMR detection [18, 19]. A joint effort will be needed to research these techniques and to create guidelines for the use and application of these. In relation to the antimicrobial stewardship programs proposed as a tool in the fight against AMR [20], veterinary microbiologists, pharmacologists, and epidemiologists as trained professionals, can ensure that these programs will succeed in medical institutions and are responsible for training future veterinary generations.

References


SESSION 4: Graduate skills and training

TRAINING VETERINARY PHARMACOLOGISTS – UPDATING THE BLUEPRINT FOR GRADUATE EDUCATION

Virginia Fajt
Texas A&M University

At the 15th AAVPT Biennial (Asilomar Conference Center, California) in 2007, a workshop was held to identify and prioritize skills and knowledge necessary for success of veterinary pharmacologists working in industry, academia, or regulatory agencies. The results were published in 2008 (V.R. Fajt, Skills and competencies required by veterinary pharmacologists: a blueprint for graduate education in veterinary pharmacology in North America, Journal of Veterinary Pharmacology and Therapeutics, 2008, 31:22-30), and the identified items are included in the appendices below.

A search of the literature since initial publication of the blueprint in JVPT in 2008 suggests that these have not been re-reviewed for graduate veterinary pharmacology. This is not surprising, since veterinary pharmacology continues to be a relatively small discipline, with few graduate training programs, whether clinical pharmacology training programs are included or not. The question then is: Is it still valid? Additional questions or items for discussion might include:

1. Would a comprehensive review of currently available graduate programs in veterinary pharmacology yield any useful data for current or potential programs?

2. Recent discussions with JVPT have clarified the scope of the journal, and they highlight the breadth of veterinary pharmacology, i.e., it may include translational work (with humans being the ultimate target) and veterinary-specific work (with animals being the ultimate target). This is part of what complicates any discussion of graduate training and skills.

3. In addition to providing students and training program leaders with a blueprint for skills, how else could AAVPT support graduate training? For example, how could industry internships be identified or supported, how could student training grants or travel grants be supported and awarded, how could emerging leaders be identified and mentored?

Appendix 1: Competencies thought to be important for careers in academia and industry, as identified during a workshop at the 15th Biennial of the AAVPT in May 2007

Nontechnical skills

Communication skills

Writing
Technical and scientific writing abilities
Basic scientific literacy (reading articles)

Formal oral presentations
Including effective Power Point presentation skills

Informal interactive communication
Able to talk up and talk down the organizational levels
Informal interactions with colleagues, lab personnel, etc.
Listening skills

**People management skills**
- Recognizing/ managing personalities
- Participate in solving problems
- Challenge and motivate

**Personal management skills**
- Time management
- Prioritization
- Organizational skills
- Task completion
- Planning/ preparatory skills
- Initiative
- Deal with success/ failure

**Technical skills**

**Study design and analysis**
- Capable of designing studies
- Ability to generate hypotheses and test them
- Knows statistical methods and has performed basic statistical analysis

**Subject knowledge**

**Understanding of clinical relevance**
- Clinical background/ ability to grasp clinical relevance of work
- For DVMs: Has clinical experience (practice), with knowledge of species-specific diseases and conditions
- Exposure to whole-animal work
- Advanced comparative pharmacology

**Research techniques**
- Able to perform needed research techniques, including chromatographic separation techniques (understanding of concepts)
- Analytical chemistry and assay validation
- Understands and can perform PD (ADME) analysis
- Understands and can perform basic PK analyses, including able to use appropriate software

**Knowledge and experience in research compliance issues**
- Exposure to GLP/ GCP
- Safety/compliance/IACUC/IRB/QA

**Additional competencies recognized as important only for careers in industry**

**Nontechnical skills**

**Personal characteristics**
- Personality fit, Culture fit
- Humility, Ability to take feedback
- Self-awareness of introvert vs. extrovert

**External focus**
- Willingness to learn and broaden horizons
- Ability to understand big picture (small companies)
Ability to focus intensely on specific details (large company)
Ability to do both (large companies)
Willing to do what is best for organization, not just project
Abilities in scenario-planning, strategic thinking, multi-dimensional planning
(including risk analysis)
Business acumen

**Interactions with others**
- Actively engage in scientific debate without getting personal
- Team player
- Consensus building
- Managing meetings
- Influence and motivate to make own decisions
- Appropriate level of delegation

**Technical skills**
- Flexibility in research area
- Exposure to industry via externships

**Additional competencies recognized as important only for careers in academia**

**Nontechnical skills**
- Grantsmanship
- Teaching experience/ concepts
- Responsibility for publication
- Increasing independence and initiative
- Employment negotiation

**Technical skills**
- Knowledge of molecular and cellular biology
WHAT INDUSTRY NEEDS FROM GRADUATES

Dan Keil DVM, PhD, DACVM
Bayer Animal Health

While not highly technical in nature, the question posed for this presentation is a difficult one and one without a straightforward answer. The complexity begins with the fact that there are multiple segments within the animal health industry and employers can be working in areas from toys and treats to food or feed additives to drugs, biologics, or pesticides. Furthermore, employers may be local or global and this difference can influence the skill sets needed to be a successful part of the organization. In our US animal health business alone, we develop veterinary pharmaceuticals (with regulatory oversight provided by the US Food and Drug Administration – Center for Veterinary Medicine), veterinary pesticides (with regulatory oversight provided by the US Environmental Protection Agency), and veterinary biologics (with regulatory oversight provided by the US Department of Agriculture – Center for Veterinary Biologics).

With that background in mind, it is easy to see how diverse the skill sets and behaviors are that can contribute positively to a career in the animal health industry. However, to provide a bit more focus for this discussion, it may be helpful to understand some of the basic requirements for the approval of a veterinary pharmaceutical product in the US. The major technical sections for a New Animal Drug Application (NADA) include Target Animal Safety (TAS), Effectiveness, Human Food Safety, Chemistry Manufacturing and Controls (CMC), and Environmental Impact. Together we will review the purpose and goals of each of these major technical sections as well as the minor technical sections (Labeling, Freedom of Information Summary, and All Other Information) to provide the audience with some idea of where their expertise and interests could apply in a product development environment.

Finally, in complex, often multi-national animal health companies, where we bring together numerous backgrounds in order to form high performance product development teams, we need tools to ensure that our associates will be in a position to succeed. One tool we use at Bayer Animal Health is to focus on competencies. A competency is an observable and measurable knowledge, skill, ability or other characteristic that contributes to successful job performance. Competencies are important because they contribute to individual and organizational success, because they can be learned, and because they can be assessed. Of particular relevance to this discussion is the application of competencies in the interviewing process. Today’s graduates can be expected to face a series of competency based questions in the interview process so that we can ensure that we are attracting, selecting and onboarding talents with the right capabilities for current and future roles.
INDUSTRY’S UNMET NEEDS

Paul Cassady, Kansas State University – Olathe
BOARD CERTIFICATION IN THE CURRENT WORLD – VETERINARY CLINICAL PHARMACOLOGY

Michael D. Apley, DVM, PhD, DACVCP
Kansas State University

The opinions set forth in this presentation are not the official stance of the American College of Veterinary Clinical Pharmacology. These are Mike’s thoughts after 32 years of being a veterinarian and 25 years as an ACVCP Diplomate.

Two preemptive points. (1) You don’t have to be boarded to distinguish yourself in clinical pharmacology. (2) A PhD isn’t a pre-requisite for doing some outstanding research. There are many among us who have demonstrated these two points. In my case, I had the opportunity to pursue a PhD and ACVCP board certification as ways of putting myself through a concentrated process of exposure, and to be evaluated according to standards of some people I greatly respect.

What is clinical pharmacology anyway?
The application of therapeutics from the view of a clinical pharmacologist may be distilled to four essential components.

• Can I do some good?
• Can I do some harm?
• Can I get it in the animal?
• What does it cost?

If you come up with something related to application of a therapeutic intervention which doesn’t fit in one of those four basic categories, let me know and I will add it.

Some measures of therapeutic effect which most completely embody the nature of clinical pharmacology to me are the Number Needed to Treat (NNT) and Number Needed to Harm (NNH).¹ These metrics express the therapeutic effect in relation to spontaneous recovery in a specific population, the specifics of which include the species (age, sex, etc.), therapeutic regimen, disease (with varying levels of detail), and possibly other specifications. All of the “Yea but…” statements and caveats that go with an NNT or NNH value are the essence of clinical pharmacology.

Description of current ACVCP diplomates
“To cure with compassion, knowledge, and diligence.”

What kind of people end up being ACVCP diplomates? Going through the 60 active diplomates on the ACVCP website resulted in some interesting statistics. They are quite an impressive group of individuals.

• Current employment
  o FDA or regulatory in another country – 3
  o Academia – 38
  o Private clinical practice or research – 5
  o Pharmaceutical company – 13
  o Laboratory or other private entity – 1
• Advanced degree beyond DVM or equivalent
  o MS – 9
  o PhD – 51
• Multiple boards – We have 17 diplomates with boards in other specialties (1 of these in two other specialties)
  o American College of Veterinary Internal Medicine – 13
  o American Board of Veterinary Practitioners (Dairy Practice) - 1
  o American College of Veterinary Anesthesia and Analgesia- 1
  o American College of Veterinary Ophthalmology - 1
  o American College of Animal Welfare – 1
  o American College of Veterinary Emergency and Critical Care – 1

Skill sets inherent in ACVCP diplomates
These are on my list of expected skillsets. I don’t think all diplomates are necessarily “expert” in all areas, but we should be conversant in the basics and able to interact with others on the team related to these areas. This list is not necessarily all-inclusive, but contains major areas of emphasis.
• A basic understanding of the drugs and therapeutics used in veterinary medicine.
• Understanding how to measure and interpret the effects of therapeutics (see clinical trials and models below)
• Understand the application and interpretation of diagnostics which lead to the administration of therapeutics. Some might argue that this aspect is out of our lane, but in the case of antimicrobial susceptibility testing, our partnership with clinical microbiologists and other specialties is critical for the appropriate application of these diagnostics.
• ADME – Absorption, Distribution, Metabolism, Excretion
  o The basics of drug movement in the body
• Pharmacokinetic modeling and integration into therapeutic regimen design, along with...
• Pharmacodynamic integration into therapeutic regimen design
• Study design, conduct, analysis, and interpretation
  o Pharmacokinetic
  o Pharmacodynamic
  o Clinical trials
  o In vitro, in vivo, and in silico models
• Inherent in the study design and analysis is an understanding of statistics along with the basic assumptions and the ability to guard against errors such as pseudoreplication, inadequate number of experimental units, unreasonable and erroneous worship of the P value, and improper analytical procedures. If these aren’t correctly designed prior to the study, the statistical analysis becomes a postmortem rather than an enlightenment.
• An understanding of Good Clinical Practices (GCP) and Good laboratory Practices (GLP). The level of competency will evolve related to amount of involvement.
• Populations. Everything a clinical pharmacologist does gets applied to populations. Sometimes the illusion of an individual animal at the point of care makes us think it is just one animal, but the only way we are of value is informing point-of-care clinicians as they work in populations of animals over the course of their careers.
• Understand the multiple facets of drug development and the regulatory framework within which this process takes place.

The most important skill sets
The skill sets above are critical, but perhaps the thought processes which can make skills and the knowledge set matter are the most important. All of the skill sets and knowledge only matter if you can...
- Apply these skills in a system, or more realistically at the convergence of multiple interactive systems, where the eventual or immediate outcome actually matters to a point-of-care clinician
  - For me these involve the animal’s physiological systems within the food animal production systems they are living and the economic environment of the client and food chain. There’s this regulatory thing too.
- Get something done
  - The need for a team is real, in fact, very little of real significance occurs outside of one. As I have gotten older I appreciate more and more that some of the benefits of the knowledge base are to be able to converse with the people much more accomplished than me in many of the skill sets mentioned above.
- Communicate the results of applying your knowledge and skills in a manner that may be efficiently utilized by the point-of-care individual.
  - Heartbreak #1 – the point-of-care person often isn’t interested in all of the gee whiz technology, modeling, or techniques you used to come up with the applicable nugget. That is why clinical pharmacologists have to split time between the practical application group and our fellow pharmacology geeks.
  - Heartbreak #2 – maybe the results of your gee whiz technology, modeling, and technical prowess are actually impractical or, gasp!, wrong at the point-of-care. That is why listening to the end users is incredibly important, the communication must be two way.

**Having an expert career as opposed to being an expert**

A favorite definition of expert is that an “ex” is a has-been and a “spurt” is a drip under pressure. My understanding of the difference in being an expert and having an expert career is based on the happy accident of signing up for a learning circle while on the faculty at Iowa State University. This experience was formative in understanding (not saying obtaining) the very nature of expertise.

The book used as the basis of the group was *Surpassing Ourselves – An Inquiry Into the Nature and Implications of Expertise*, by Bereiter and Scardamalia. The direction is set in the early part of this book when the authors describe an Air Force study on why some fighter jet technicians were so much faster and efficient than others. They note that the findings demonstrated “the experts were not superior in general problem-solving skills or in knowledge of electronics, and they went about trouble-shooting in the same way as the less expert technicians. But the experts differed from the nonexperts in having a very deep understanding of the particular systems they were working with.”

In defining the general nature of expertise, Bereiter and Scardamalia noted “Our conjecture is that in order to be experts, people must choose to address the problems of their field at the upper limit of the complexity they can handle.” And, “For the individual, a method of expertise would consist of personally effective and rewarding ways of living at the edge of one’s competence...”.

Perhaps the most helpful part of their book is in defining this “expert career”. Bereiter and Scardamalia emphasize that an expert career need not be specialized. Rather, “What makes it an expert career is that it is pursued in the manner of...addressing and readdressing, with cumulative skill and wisdom, the constitutive problems of the job, rather than reducing the dimensions of the job to what one is already accustomed to doing.” Board certifications and graduate degrees can lead to two outcomes. One is the expert, constantly evolving to know more and more about less and less. Or, the other is to use these endeavors as the starting points for competence to support an expert career. Expert is something you do, not something you have.

Perhaps a most informative way to assure that we continue on an expert career path is to set aside time once a year to read the famous paper entitled *Unskilled and Unaware of it: How Difficulties in Recognizing One’s own Incompetence lead to Inflated Self-Assessments*, and the follow up paper, *Why the Unskilled are Unaware: Further
**Explorations of (Absent) Self-Insight Among the Incompetent.**³,⁴ These papers support the concept that an expert career is based on a keen sense of what is not known, by both the individual and the collective expertise of the discipline.

**What should we be doing?**
Evidence-based medicine (EBM) must have multiple homes. EBM was described way back in 1997 by Sackett, et al, in their book *Evidence Based Medicine – How to Practice and Teach EBM* as having the three basic components of 1) the best research evidence, 2) clinical expertise, and 3) patient values.⁵ (Referenced is my dog-eared copy of their second printing, with newer editions available.) The category of patient values translates to veterinary medicine in the consideration of client values along with the needs and best interests of the patient.

It makes sense that the therapeutics portion of EBM is supported by the basic skill sets described above for a clinical pharmacologist. I believe that an inherent responsibility of ACVCP diplomates is to cast light on the evidence or lack of evidence supporting application of therapeutics in veterinary medicine. Another responsibility is to evaluate both internal and external validity of the evidence available, which is dependent on being able to understand all aspects of the design, conduct, and analysis of these studies.

It is also a responsibility to understand and lead in the application and interpretation of some diagnostics which result in the administration of therapeutics. For example, what should be done when a “diagnostic” for mastitis therapy becomes available which applies antimicrobial susceptibility testing to mixed cultures of unknown genera and species?

This leadership must not only be displayed in interaction with individual point-of-care clinicians, but also within professional organizations and in relationships with regulatory agencies and legislative bodies.

The diverse strengths of ACVCP diplomates are being applied in all of these areas, bringing to mind many of our individual diplomates and their extensive contributions in their areas of focus. Perhaps learning of discoveries and techniques from other focus areas within clinical pharmacology is one of the biggest supporting mechanisms for advancements in one’s own focus area. Looking at the current ACVCP diplomates, it is clear that graduate degrees and boards were just the start of expert careers devoted to expanding their personal knowledge and capabilities, with animals and society benefitting along the way.

**References**

SESSION 5: Start-ups and New Business Ideas

Recent Trends in Patent and Trade Secret Laws and Their Impact on Our Industry

Michael Annis, Partner, Husch Blackwell LLP

The principal purpose of this presentation is to address with the audience growing trends in the intellectual property world, focusing on patent and trade secret rights, and, in particular, how those trends are impacting the animal health and biotech industries. Changes in patent law over the last decade, especially in the area of patent eligibility, along with enactment of the Defend Trade Secrets Act, has forced a rethinking of how to protect innovation and advancements in biotechnology and diagnostic methods. The program will address the changes in law and how to better navigate the new paradigm. We will also discuss pending federal legislation addressed to the American patent system and whether we may see some normalization on the issue of patent eligibility. I will unpack recent case law and pending litigation to reveal industry vulnerabilities, while offering a look ahead to the likely direction future disputes will take.

Michael Annis is a St. Louis-based attorney with decades of experience protecting the corporate value of animal health and agribusiness clients, including claims of, intellectual property infringement and enforcement, misappropriation of trade secrets and confidential business information, and false advertising. A 25 plus year veteran, Michael has litigated and tried hundreds of these cases in federal and state courts, as well as before a number of federal administrative agencies.

Michael is a frequent speaker on a number of topics within the intellectual property world including on patent, trade secret protections, and associated litigation.
I HAVE THIS IDEA, NOW WHAT?

Hank Mills

Oleander Medical Technologies
WHERE DOES THE MONEY COME FROM?

Shawn Glinter

Pendant Biosciences
TRANSFERRING TECH OUT OF A UNIVERSITY

Troy Brady

Auburn University
TRANSFERRING TECH INTO A COMPANY

Scott Brown

Zoetis
Meeting Registrants
Melissa Andrasik, Dechra Development
Michael Annis, Husch Blackwell LLP
Michael Apley, Kansas State University
Dawn Boothe, Auburn University
Troy Brady, Auburn University
Bonnie Bragdon, Zomedica
Scott Brown, Zoetis
John Byrd, Southwest Bio-Labs
Jennifer Caldwell,
Michela Cantiello, Avogadro LS
Clelia Chauvin,
Cynthia Cole,
Ana Costa,
Jennifer Davis, Virginia-Maryland Regional College of Veterinary Medicine
Keith DeDonder, Veterinary and Biomedical Research Center, Inc
Dubraska Diaz-Campos, The Ohio State University
John Donecker,
Virginia Fajt, Texas A&M University
Alyson Fitzgerald, Kansas State University College of Veterinary Med
Ronette Gehring, Utrecht University
Shawn Glinter, Pendant Biosciences Inc
Joe Gloyd, Elevate DVM, Inc.
Sophie Gretler,
Briana Hamamoto-Hardman,
Jonathan Hare, Telemark Veterinary Consulting Inc.
Joanne Haughan, University of Pennsylvania
Mark Heit,
Klaus Hellmann, KLIFOVET AG
Katie Hope, Merck Animal Health
Matthew Hull, University of Illinois Veterinary Teaching Hospita
Rob Hunter, One Medicine Consulting
Inga Karasek, St. George’s University
Shane Karnik, Pyxant Labs Inc
Dan Keil, Bayer Animal Health
Heather Knych, School of Veterinary Medicine, UC Davis
Butch Kukanich, Kansas State University CVM
Chantal Lainesse, SterileCare Inc
Jeffrey Lakritz, The Ohio State University
Cory Langston, Mississippi State University
Zhoumeng Lin, Kansas State University
Susan Longhofer, Dechra Pharmaceuticals
Anthony Lucas, Dechra
Geraldine Magnin, Kansas State University
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Travis Mays, Texas Veterinary Medical Diagnostic Laboratory
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