2007 Summer Leadership and Research Program Abstracts

The following abstracts were prepared by students participating in the 2007 Summer Leadership and Research Program at the Ontario Veterinary College.

**Genetic contributions to atrial fibrillation in standardbred horses**

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In Atrial Fibrillation (AF), the most common clinically relevant arrhythmia in horses, the atria contract in a random disorganized manner resulting in an irregular cardiac rhythm. Affected horses cannot reach maximum cardiac output, and perform poorly. AF occurs quite commonly in Standardbred horses, with a frequency indicating probable polygenic genetic contributions. Further, among AF horses presented at the Ontario Veterinary College, a significant proportion involves a specific bloodline, suggesting simple Mendelian inheritance in this subgroup. The objectives of this study are to assess genetic contributions to AF in Standardbred horses and to analyze possible inheritance patterns. Our null hypotheses are: 1) comparison between disease positive and presumptive disease negative populations will reveal no evidence of genetic contribution 2) comparison between bloodline-specific disease positive and presumptive disease negative populations will reveal no evidence of genetic contribution and 3) distribution of the disease trait within the bloodline of interest will reveal no evidence of Mendelian inheritance. Our methods include genetic analysis of the full Standardbred breed registry database to assess the heritability of AF, and genetic analysis of affected horses to determine inbreeding coefficients and genetic variance. Using a model under development in a parallel study, all confirmed disease positive horses’ racelines will be analyzed to characterize the performance pattern of arrhythmia onset and recovery. We will then look for this pattern in the racelines of all horses in the bloodline of interest in an attempt to identify disease positive horses that were not presented at the OVC. The overall proportion of the bloodline identified as disease positive by this test will be used to further evaluate inheritance patterns. If we confirm that there are genetic contributions to AF in the horse, we can pursue underlying mechanisms, including the development of a genetic test to screen for predisposition to the arrhythmia. This will be of benefit to the Standardbred racing industry.

**Neurogenesis of stem cells isolated from equine umbilical cord blood**

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Here, we report for the first time that equine umbilical cord blood derived stem cells (eCB-SCs) with proven mesenchymal potency can be induced toward neuronal-like cells. Neurogenic induction was performed in serum free (SF) media supplemented with retinoic acid (RA) and brain-derived neurotrophic factor (BDNF) for 10 days. Morphological assessment and immunocytochemistry assays revealed positive neuronal induction in two separate eCB-SC lines. Neurogenesis was supported by strong positive staining of the early neuronal protein markers beta-tubulin III and glial fibrillary acidic protein (GFAP) marker as well as weak positive staining of the mature neuronal marker microtubule associated protein 2 (MAP2). No morphological changes were seen in induced equine dermal fibroblast (eDF) lines although positive immunocytochemistry staining was observed. These results suggest that RA and/or BDNF are effective neurogenic inducers of eCB-SCs. We conclude that eCB-SCs potency may not be restricted to mesenchymal lineages, but eCB-SCs may have multipotent potential.

**Bartonella spp. in the blood of raccoons and rodents in southern Ontario**

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*Bartonella* spp. are gram-negative bacteria that live inside red blood cells and cause prolonged bacteremia in their hosts, as well as being implicated in zoonotic infections. Immunocompromised people are particularly at risk since infection may prove fatal. *Bartonella* spp. have been identified in rodent populations, but not yet in raccoons. Blood from raccoons and rodents in southern Ontario was plated on nutritionally-rich ‘chocolate’ agar, the most widely used method for the microbiological diagnosis of bartonellosis. Twenty of 40 rodent samples were culture positive for *Bartonella* spp., but none of 20 raccoons tested positive. A PCR based assay amplifying the citrate synthase (*gltA*) gene of *Bartonella* spp. and sequencing of the PCR
product was done on a 14 rodent isolates. Nine out of 14 bacterial isolates from rodent blood were identified as Bartonella spp. and alignment of these sequences showed a high degree of homology, but 3 of these isolates showed slightly greater divergence. This study suggests that raccoons may not carry Bartonella spp., however this study was done on this small population of raccoons in a limited geographical area.

Cloning of porcine complement C3d as a potential vaccine adjuvant for neonatal pigs
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Vaccination is a widely used strategy in maintaining swine herd health. Nevertheless, providing protection via vaccination remains a challenge with neonates due to interference by maternal antibodies. C3d, a fragment of complement component C3, has been shown to increase the immunogenicity of an antigen in mice by up to 10,000 fold. This research aims to clone and modify porcine complement C3d, for eventual use as a vaccine adjuvant. The poC3d gene fragment was successfully extracted from two boars and sequenced. The samples showed a high degree of homology with the published poC3d sequence, as well as two consistent mutations throughout the samples. Interestingly, one of these mutations resembles the wildtype sequence of humans, sheep, cattle, mice, guinea pigs, and rabbits. One of the samples shows promise for eventual use in a vaccine construct, with expression of this particular gene fragment as a protein remaining a future objective.

A study of heart rate and rhythm during live racing in the Standardbred racehorse
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Poor performance and sudden death are sources of concern for the horse racing industry. To determine what role cardiac dysfunction may play, we need greater insight into cardiac events occurring during live racing.

A simple monitoring system has been developed to obtain an ECG during regular competition. It consists of a Holter monitor recording device and a custom designed girth. The adjustable, lightweight girth has built-in electrodes and fits unobtrusively beneath the regular racing harness. Electrodes are constructed from electrically conductive, pliable elastomer, and require neither clipping nor use of electrode paste. The girth, which is applied in the paddock during harnessing to avoid disrupting regular routines, remains in place during both warm-up and racing and allows acquisition of diagnostic quality ECG signals during all phases. Participation in the study is a condition of designated races; i.e., all horses in each race are fitted with the system.

Data collection is ongoing. Results to date reveal that horses rarely drop beats in the paddock, in contrast to normal resting behaviour, and heart rates during racing can reach 270 bpm. Variations in heart rhythm have been detected and are indicative of the physiologic demands of racing. Horse size, conformation, gait, stride characteristics, harness style, and trainer harnessing preferences can all affect signal quality.

The initial objective is extraction of heart rate and rhythm to establish normal values during racing. Further analysis will then investigate exercise-associated changes in ECG waveforms.

Non-invasive surveillance of cannon bone and joint health in racehorses by quantitative ultrasound velocity and biomarkers
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Condylar fractures and articular cartilage degeneration are a significant problem in the equine industry. These injuries are the acute manifestation of a chronic problem related to non-adaptive changes in subchondral bone, and often result in the premature retirement or euthanasia of racehorses due to catastrophic breakdowns. Previous research in cadaver racehorses has shown a high incidence of joint disease and non-adaptive changes in subchondral bone putting a significant number of horses at risk of failure. This study plans to monitor cannon bone and joint health in 65 actively racing 2 and 3 year old Thoroughbreds over 2 racing seasons. Quantitative ultrasound will be used to monitor third metacarpal bone quality. This device creates pulsed acoustic waves, where the sound waves’ shortest propagation time through the bone is calculated to determine the speed of sound (SOS) at specific regions. Serum biomarkers will also be used to monitor bone and cartilage anabolic and catabolic activity in response to exercise. As a surrogate measure of loading, we will relate our SOS and biomarker findings to a cumulative work index, which is a means to evaluate exercise intensity considering distance and speed. This study hopes to increase knowledge of the response (rate and magnitude) of bone and cartilage to exercise. This should help towards the development of a non-invasive method of detecting early changes in subchondral bone indicative of future failure. The realization of these goals could eventually lead to the implementation of preventive training programs to minimize wastage in the racehorse industry.
A review of the welfare implications of blistering and pin firing in horses
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Pin firing and blistering are forms of a therapy known as counter-irritation, used to treat soft tissue injuries in horses. Pin firing refers to a technique in which red-hot metal pokers (firing irons) are applied to the skin of the affected area, usually a lower limb. Blistering involves application of a blistering agent to the affected leg, where it produces chemical burns. These agents are composed of highly corrosive and irritating substances including mercury, iodine and acetone. Treated horses must be restrained while the blister is present to prevent ingestion or transfer to other body parts. Pin firing and blistering date back to the 1700’s, and have received little scientific scrutiny. While the procedures are believed to improve healing through induction of inflammation and the resultant increase of blood flow to the area, this positive effect has not been scientifically proven and current knowledge concerning tissue dynamics contradicts this core belief. Benefits may result indirectly due to enforced rest rather than the counter-irritation itself. Welfare issues associated with the procedures include acute and possible chronic pain, restriction of natural behaviour resulting in frustration, and possible malaise due to infection or toxicity. Although anecdotal information suggests the use of such practices is continuing, prevalence data are lacking. The AAEP released a position statement on the practices of pin firing (2006) and blistering (2003). The AAEP acknowledges that these procedures are painful and potentially dangerous, but condones treatment for certain undefined injuries, when used in combination with analgesics to control pain. The CVMA, however, is opposed to firing due to the lack of evidence of efficacy required to justify the amount of pain and tissue damage endured. As an alternative to counter-irritation, icing and bandaging the limb in combination with strict stall rest, may be considered. In addition, therapies such as Cryosurgery/Cryotherapy (liquid nitrogen application), Stem cell injection, and Shockwave Therapy (possibly combined with stem cell injection) are also increasing in popularity. To date, however, the efficacy of these procedures has not been adequately examined. The goal of this review is to raise awareness of the welfare issues associated with pin firing and blistering. Given the controversy surrounding these practices, guidance is needed to assist veterinarians with decision making and advising equine clients regarding such practices. Further research examining welfare implications of counter-irritation is needed so that these methods of treatment can be either rejected scientifically or modified to ensure humane treatment of horses undergoing the procedures. In particular, equine practitioners should be informed about valid protocols for pain management when performing these procedures to ensure compliance with the AAEP guidelines.

The effect of retention on performance of Standardbred racehorses: A pilot study
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The use of performance enhancing drugs is a contentious issue in equine sports. In Ontario, drug use is regulated by the Ontario Racing Commission (ORC) and drug testing is performed by the Canadian Food Inspection Agency (CFIA). Recently some racetracks in Ontario have independently implemented use of retention stalls to control drug use. Horses assigned to these stalls must arrive 24 hours prior to the race where contact with people and other animals is restricted, veterinary assistance is monitored and access to turn out may be limited. Retention stalls are constantly monitored to ensure all regulatory measures are observed. Some trainers and owners express concerns regarding the impact of retention barns on performance, independent of performance enhancing drug use. Novel housing and social environments, as well as disruptions to routine husbandry, can result in high stress levels for some horses. Research indicates that the pre-race period is important to performance, demonstrating that relaxed or quiet horses are more likely to win. The objective of this pilot study is to identify effects of retention on performance by examining the race records of horses racing out of retention in comparison to performance of all other starts. The population under investigation is a convenience sample of Standardbred horses raced out of retention in Stakes races on Woodbine Entertainment Group (WEG) tracks from 2005 to July 27, 2007. A retrospective performance analysis was performed on each horse using the horse’s lifetime performance record. Using course graphical and statistical analysis, no significant effect of retention on the outcome variables (individual racetime, individual last quarter time) examined has been demonstrated that can not be explained by other race factors, such as overall racetime. The data will be further subjected to a more sensitive statistical model designed to control for the variety of factors previously demonstrated to affect race outcome. Based on the results of this study, we expect to make recommendations to the ORC for further investigation.

Teaching with virtual microscopy
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Virtual Microscopy is of the newest and most innovative technologies used in pathology today. It has already been established in clinical and research settings across the world and has started its invasion into the world of education. Virtual microscopy is the process of converting an entire glass slide into a high-resolution image. Scanner systems use actual microscope objectives to scan in the traditional glass slide. Much more than a photograph of a slide, the image can be viewed at any common microscope magnification along...
with any intermediate magnifications one should choose. The scanned image can be projected on any desktop computer with internet access, thereby allowing one to view the slide without the use of a microscope.

There are many benefits to using virtual microscopy in an educational environment. Virtual microscopy removes the need to create and store entire class sets of slides. Only one slide needs to be scanned and each student is able to view the slide, at the same time, on a monitor. Rare slides will no longer be kept in storage for fear of damage but now can be shared by everyone. Virtual microscopy will cut down the costs of not only preparing new slide sets but also the cost of maintaining entire microscope labs.

Virtual microscopy in not meant as a replacement for traditional microscopy but instead to enhance the entire microscopy experience. Allowing students to become comfortable using both traditional microscopes and the newest technology will be of great importance as they move into the work force. It can be integrated into a classroom setting in a variety of ways by allowing great flexibility for both different teaching methods and classroom layouts. The free viewing software allows the instructors greater control and interaction between slides and students.

The Departments of Biomedical Sciences and Pathobiology have recently purchased the ScanScope® CS System from Aperio Technologies. Scanning and teaching with the new digital slides is expected to begin in the new school year within a few histology tutorials and pathology labs. Students will be able to view and study these slides through links on Blackboard (WebCT) through a free downloadable program called ImageScope™. Within the next few years both departments plan to make virtual microscopy a key component in the curriculum.

**Effectiveness of using a non-penetrating captive bolt for on-farm euthanasia of low viability piglets**

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Low birth weight piglets have a low survival rate and often must be euthanized. Manual blunt trauma to the head is currently recommended by the AVMA. A non-penetrating captive bolt device (Zephyr) that is effective and more aesthetically acceptable would be a valuable alternative. The objective of this experiment was to determine the effectiveness of the Zephyr in comparison to blunt trauma for on-farm euthanasia of low viability piglets. Seven stockpeople from four commercial and one research farm were supplied with a Zephyr gun and air compressor and trained how to operate the device. Stockpeople euthanized low viability piglets with either the Zephyr (ZE) (N=48) or manual blunt trauma (BT) (N=43). Signs of sensibility, respiration, duration of reflex movements and heart beat were recorded. Subcutaneous and subdural hemorrhaging and degree of skull fracture were scored on a five point scale during post-mortem examination. Piglets euthanized by ZE had longer durations of leg movement and heart beat (150.4 ±16.9 vs. 78.05 ± 10.1/sec and 481.3 ± 50.3 vs. 167.2 ± 23.8/sec, P<0.0001, respectively; Proc Mixed) as well as higher subcutaneous and subdural hemorrhage scores (3.7 ± 0.15 vs. 2.25 ± 0.2/sec, P<0.0001 and 3.7 ± 0.15 vs. 2.63 ± 0.24/sec, P=0.0006; respectively) as compared to BT regardless of stockperson performance. However, there was also variation among stockpeople for skull fracture score (P<0.028) and a trend with respect to subcutaneous hemorrhage score (P<0.07) suggesting the degree of trauma is inconsistent. There were a greater number of ZE piglets (10/48) which regained signs of sensibility as compared to BT (0/43). Manual blunt trauma to the head is a rapid and effective method of euthanizing low viability piglets. Further modification to training, technique and the apparatus are required before the Zephyr can be recommended as a humane option for euthanasia.

**Evaluation of an automated System and comparison with traditional methods of estrus detection in Holstein heifers**

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The efficiency of a dairy operation relies on timely and successful breeding of heifers in order to remain economically competitive. Heifers are known to be more fertile and better demonstrate estrus behaviours when compared to cows. The challenge remains for producers to effectively manage heat detection and the subsequent breeding of animals despite their busy schedule. This study described and evaluated the accuracy of an automated heat detection system in identifying animals that are in estrus. The Heatime heat detection system (Lely Group and SCR Engineering Inc) is a stand alone, automated activity monitor. An acceleration sensor, microprocessor and memory are used to accumulate data on the speed of movement as well as restless behaviour. A proprietary algorithm analyzes data relative to an index based on individual data from the previous 7 to 10 days. One hundred one Holstein heifers were enrolled at a mean age of 14.7 months, ranging from 13.8 to 16.0 months. Pregnancy was diagnosed using rectal palpation and ultrasound examination not earlier than 28 days after insemination. The study included three cohorts (group 1, n=34; group 2, n=35; group 3, n=32) of Holstein heifers. Each of the first two groups were in the monitored breeding pen for approximately 6 weeks, and the third group was observed in the pen for 37 days. The animals were monitored by the automated system and animals were bred once daily, in the morning, on the basis of the system. The conception risks for animals with
confirmed pregnancy outcomes were 77%, 81%, and 67% for groups 1 to 3, respectively. In the third group, the accuracy of the automated system was evaluated by measuring serum progesterone levels, with 83% of the animals identified correctly by the system as being in estrus (serum progesterone < 1.0 nmol/L). The agreement between the Heatime system and visual observation of estrus and Kamar heat mount detectors was moderate (kappa = 0.4) and poor (kappa = 0.3), respectively. Additionally, the intensity of estrus detection with the automated system was 86% when compared to the heat mount detectors and 57% compared with observation of standing heat. The system appeared to produce only 1-2% false positives using observation of standing estrus or secondary signs (including standing estrus) as the gold standard, respectively. The system appeared to identify animals in estrus, with good conception rates, while producing few false positives.

An investigation of the prepartum behaviour of primiparous and multiparous dairy cattle
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The impact of parity and its effects on the behaviour of dairy cattle in relation to calving difficulty and associated pain has yet to be determined. The objective of this double-blind study was to determine the behavioural differences between eight primiparous and 13 multiparous dairy cattle in the immediate preparturient phase. Previously recorded videotapes of the 24 hours prior to calving were analyzed. Data collection involved video scan sampling every 5 minutes to analyze eating, drinking, periparturient and pain-related behaviours, calving difficulty, and the use of a datalogger to record standing and lying activity. Calving difficulty was scored as: 0-unassisted, 1-easy pull, 2-hard pull, 3-cesarean section. Data was analyzed using StataSE 10 to conduct mean comparison univariable analyses. Preliminary results suggest heifers have more drinking episodes and have more head-to-flank contact events while standing than do cows. (0.027 vs. 0.015, p=0.04; 0.034 vs. 0.014, p=0.007 respectively). Heifers requiring assistance at calving also drank significantly more than heifers with unassisted calvings, p=0.04. Results also suggest heifers requiring assistance at calving are lying significantly longer than cows with assisted calvings (868 min/day vs. 555 min/day, p=0.01). Cows with more difficult calvings (C.S. ≥ 2) were lying down for significantly less time throughout the day however the number of lying bouts experienced during this time was almost equal to that of cows experiencing an easier calving (C.S. < 2) (6.15 hr/day vs. 10.98 hr/day, p=0.05). It is possible that these behavioural differences are a result of underlying discomfort associated with parturition and/or a response to this novel experience in primiparous cows. There may be instances where pain management at calving is warranted or behavioural cues are used to predict difficult calvings.

An evaluation of the Brix Refractometer for measurement of colostrum quality and success of passive transfer
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The objective of this study was to evaluate the practical usefulness and diagnostic test characteristics of the Brix refractometer, in comparison to gold standard laboratory tests, for measurement of immunoglobulin content of bovine colostrum and success of passive transfer in dairy calves. A total of 132 cows, and 202 newborn Holstein calves were enrolled in this study. Cow colostrum samples were taken at the time of first feeding to each calf, and blood samples were collected from each calf between 1 and 4 days of age. Colostrum samples were evaluated by the Brix refractometer for immunoglobulin concentration at 5, 20 and 38°C. Temperature had little effect on the Brix readings, with a mean difference between temperature groups of 0.2 Brix % units. In comparison to radial immunodiffusion assay results, the Brix readings had a moderate degree of correlation (R²=0.4). Mean serum total solids (TS) as measured by digital refractometry was 53.4g/L (+/- 0.45), whereas the mean serum TS as measured by the Brix refractometer was 8.8 (+/- 0.06) Brix % units. These values had a high correlation value at R²=0.65. Results of this study suggest that the Brix refractometer may be a useful on-farm tool for colostrum management. However, in order to more fully evaluate the diagnostic test characteristics of the Brix refractometer, a larger sample size of cow colostrum and calf serum having greater variability in quality, and degree of serum TS is recommended.

Multiple anthelmintic resistance in an Ontario sheep flock
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Gastrointestinal nematodes (GIN) in sheep cause anemia, edema, and diarrhea, with associated weight loss, poor milk and wool production, and reduced fertility. Broad-spectrum anthelmintics reduce GIN levels, but anthelmintic resistance (AR) has become a worldwide problem. Under dosing and/or excessively frequent dosing are two means of selection for AR. There are signs of AR in Canada but no study has proven its existence. A farm with a history of poor response to ivermectin and albendazole was selected for a fecal egg count reduction test. Sixty ewes were divided into 4 anthelmintic treatment groups (ivermectin, albendazole, moxidectin, and levamisole) and a control group. A fecal
Characterization of equine serum lectins that recognize bacterial pathogens

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Collectins and ficolins represent two collagenous lectins which contribute significantly to innate immunity in young mammals such as humans, mice, and pigs. Furthermore, single nucleotide polymorphisms (SNPs) have been found in the DNA coding for these proteins leading to functional deficiencies which play a critical role in the difference in pathogenesis of disease between animals. As the importance of these proteins in altering the susceptibility of certain animals to disease becomes more apparent, the presence of these proteins in the equine species have yet to be characterized. Therefore, the objective of this project is to determine which innate defense proteins are present in equine that provide protection against bacterial pathogens. To date, bacterial binding assays and High Performance Liquid Chromatography (HPLC) techniques each followed by SDS-PAGE gels and Western immunoblot have been tested. Preliminary data suggest that proteins in the molecular weight range of ficolin may be present in the serum of horses using HPLC. Peptide mass fingerprinting and amino acid sequencing via mass spectrometry (MS/MS) will help confirm the expression of ficolin and any other innate defense proteins. The long-term objective of this project is to gain a better understanding of innate immunity in horses by characterizing equine lectins. This will be done using protein and DNA analysis in relation to the pathogenesis of infectious diseases between individual and families of horses.

Zoonotic diseases and infection control for public health professionals and pet owners

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It was brought to our attention that there is currently a lack of knowledge regarding zoonotic diseases among pet owners as well as in the medical community. Information sources are not sufficient for the purposes of physicians, veterinarians, and public health professionals, they are few in number, and data is not easily accessible. A resource that was accessible, coherent, relevant, thorough, as well as addressed the specific needs of these different individuals, was in need.

The purpose of the project was to increase the public’s knowledge of zoonotic diseases by creating resources for veterinarians, physicians, public health professionals and pet owners. Information packages were made for different common household pets and consisted of information on prevalence and incidence, the geographic distribution of infections, who is most at risk and risk factors, the mode of transmission, human symptoms, and signs in animals.

Factors that needed to be considered included the structure, format and presentation of the information itself, the effectiveness of the presentation of the information, and to determine what type of information was most valuable and to whom. Challenges included a lack of information regarding the occurrences of zoonotic diseases, the fact that not all zoonotic diseases are reportable and that not all reportable zoonotic diseases are diagnosed or even detected.

There are inherent risks that will always be associated with pet ownership. However, by developing good informational materials, we hope to increase the awareness of potential zoonotic diseases associated with pets and the ways in which to reduce these risks, thus reducing the occurrence zoonotic infections. With a broader base of knowledge, pet owners, physicians, public health professionals as well as vets can more easily work together in order to reduce the transmission of zoonotic diseases in the household. For this reason, a network of professionals in the fields of veterinary medicine, human medicine, public health and infection control are currently being established in order to contribute to the success of the project.

The project is still in its beginning stages; however the work done during the summer of 2007 has provided a solid basis of research and information to be used in the future as the project progresses.
Studies by Undergraduate Researchers at Guelph
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**Uterine Proteins Involved in Failure of Early Pregnancy in the Mare**


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After entry into the uterus, the equine embryo becomes enveloped by a polysaccharide-rich capsule, which is modified and lost during the third week of gestation. Embryonic loss is of significant economical importance in horse breeding and most of the losses occur during the period when the capsule is present.

Previous work has showed that after luteolysis with PGF2alpha, the amounts of a protein identified as a secretory phospholipase A2 (sPLA2) increase markedly in embryonic capsules in all mares examined at Days 17 and 18. By comparison, normal pregnant mares secrete sPLA2 in low amounts that is not detectable in normal uterine flush fluids. The objective of this study was to characterize the role of sPLA2 and uteroglobin in pregnancy failure, mainly by using polyclonal antibodies to synthetic peptides of equine sPLA2 and uteroglobin. Uterine lavage fluids, endometrial biopsies and intact encapsulated embryos were collected from approximately 8 treated and 8 control pregnant mares between days 15 and 25 post ovulation.

Our results have shown after luteolysis with PGF2alpha sPLA2 increases markedly in all mares examined at Days 17 and 18. It was also noted that uteroglobin, a protein with unknown function but is abundant in uterine secretions, increased in the normal uterine fluid in treated mares.

These dramatic changes in sPLA2 and uteroglobin in association with the failure of pregnancy suggest that immunoassays for these might have diagnostic use. Also, based on known activity of sPLA2, inhibitors might become useful for treatment of infertile mares.

**The effect of three thawing protocols on equine semen quality**

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Past studies have shown that the method used to thaw semen has great impact on semen quality. Studies tested the effects of three thawing protocols on three measures of post-thaw semen quality. Optimal thawing procedures that resulted in the best post thaw semen quality were sought. Semen samples were collected from eight stallions and frozen using an accepted method. Each stallion’s semen was thawed in a water bath using three protocols: 37°C for 30 seconds, 38°C for 10 seconds and 75°C for 7 seconds. Samples were then analyzed for sperm motility, morphology and viability/acrosome status. Total and progressive motility was measured immediately and one hour post thaw using Computer Aided Sperm Analysis. Morphology was measured using eisin-nigrosin stain and viability/acrosome status was measured using dual fluorescence staining and ultraviolet light microscopy. Motility was highest using the 38°C/10sec method, morphology was superior using the 37°C/30sec method and viability/acrosome status was superior using the 75°C/7sec method. Despite adequate post-thaw motility, viability results for some samples were markedly lower, suggesting technical problems with staining. There was also an individual stallion effect. Statistical significance is yet to be determined. It can be concluded that thawing method affects semen quality; however, many methods result in viable sperm. Further research is required to investigate the contradictory results and to better understand the impacts of semen thawing.

**The Tei Index: A new parameter to identify occult DCM and overt DCM Dobermans?**

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BACKGROUND: Dilated cardiomyopathy (DCM) results in dilation of one or both ventricles with subsequent circulatory dysfunction. Early detection is important since therapy can prolong the preclinical phase. The Tei index, which is defined as the sum of isovolumic contraction time and isovolumic relaxation time divided by ventricular ejection time, may offer improved diagnostic and prognostic capabilities since it addresses both systolic and diastolic phases of the heart cycle. OBJECTIVES: The objectives of the study were to determine the utility of the Tei index in discriminating between Dobermans with DCM (occult or overt) and Dobermans without DCM, and to determine whether the Tei index could predict time to onset of overt DCM and survival time in Dobermans with occult and overt DCM, respectively. METHODS: 50 dogs were retrospectively selected for each group (normal, occult DCM, overt DCM) based on echocardiographic measurements and clinical presentation. The Tei index was calculated for each dog and compared between the three groups. RESULTS: Dobermans with occult DCM had a significantly higher Tei index than normal dogs (p=0.0009) and dogs with overt DCM (p=0.0003), but the Tei index of normal Dobermans and those with occult DCM did not differ (p=0.744). The Tei index did not predict time to onset of clinical signs or sudden death (SD) in the dogs with occult DCM (p=0.635), nor did it predict time to death in dogs with overt DCM (p=0.064). CONCLUSIONS AND CLINICAL IMPORTANCE: The Tei index can differentiate Dobermans with occult DCM from normal Dobermans and those with overt DCM. The Tei index is not a useful predictor of prognosis in Dobermans.
**Effects of storage time and hemodilution on canine platelet function**

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The ability to evaluate primary hemostasis can provide insight into the pathophysiology and management of a number of diseases that impair platelet function in dogs such as immune-mediated hemolytic anemia, immune-mediated thrombocytopenia and disseminated intravascular coagulation. Aggregometry is considered the gold standard assay for assessing platelet function; however, its clinical utility is limited. The Platelet Function Analyzer-100\(^\circledR\) (PFA-100) is a bedside point-of-care test, but its clinical utility remains to be tested thoroughly. According to manufacturer’s guidelines, the PFA-100\(^\circledR\) requires hematocrits (Hct) greater than 0.35L/L and samples to be analyzed within 4 hours of collection, which can present logistical problems in the clinical setting. Objectives of this study were: i) Establish effects of different storage times on platelet function as determined by PFA-100\(^\circledR\) and aggregometry; ii) Establish effects of hemodilution on PFA-100\(^\circledR\) closure time (CT); and iii) Establish if addition of autologous packed red blood cells to anemic blood corrects PFA-100\(^\circledR\) CT. PFA-100\(^\circledR\) closure times were not significantly different at times 0, 90, 120, 240 and 360 min (P=0.20). Similarly, aggregometry did not show any significant changes in maximum aggregation and initial velocity of aggregation among comparisons at 90, 120, 240 and 360 min (all P values >0.05). Hemodilution of blood with autologous platelet-rich plasma resulted in significantly different PFA-100\(^\circledR\) CTs among whole blood, 0.35L/L, 0.25L/L, and 0.15L/L hematocrit values (P<0.05). The addition of autologous packed red blood cells significantly lowered the CT (P<0.01) and restored it into the reference range. We conclude that: i) Sample storage time of up to six hours does not significantly alter platelet function as assessed by PFA-100\(^\circledR\) and aggregometry testing; ii) Serial hemodilutions result in prolongation of PFA-100\(^\circledR\) closure time; and iii) Addition of autologous packed red blood cells into hemodiluted samples resulted in restoration of PFA-100\(^\circledR\) closure times to within reference intervals.

**Proteomic analysis of archived pathology tissue**

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Pathology archives consisting of vast numbers of formalin-fixed paraffin-embedded (FFPE) clinical specimens are a promising resource for discovery driven proteomic research in investigative pathology. The common practice of formalin-fixation impedes proteomic analysis due to protein-protein and protein-nucleic acid cross-linking. The short-term objective of this project is to develop and optimize a method for extracting proteins from FFPE tissues and to analyze the quantity and quality of recovered protein. This work will then be applied to the investigation of differential protein and peptide expression in the epidermis of healthy and diseased canine skin. To extract proteins from fixed tissues, sections were deparaffinized and heat treated in lysis buffer. In addition, canine epidermis was isolated by manual microdissection. Protein extracts were characterized by 1D and 2D PAGE as well as Liquid Chromatography tandem mass spectrometry (LC-MS/MS). So far, a method has been optimized to extract proteins from paraffin-embedded tissues. Initial results show distinct proteins on 1D PAGE and 2D PAGE for tissues fixed in formalin. Over 30 proteins have been identified in canine epidermis by LC-MS/MS and have a range of functions including structure, motility, gene expression and energy production.

**Prophylaxis of food allergy by designer probiotics**

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With the current human food allergy epidemic, it is desirable to investigate means to direct the immune response away from predisposition to food allergy. Previous research here has described methods to induce allergy to the major food allergen, ovomucoid (Ovm), in outbred neonatal pigs. This model was used to investigate preventative treatments for food allergy to Ovm with possible relevance to human food allergy. We hypothesize *Escherichia coli*, with or without interferon gamma (IFN-γ) delivered intramuscularly, can act as a probiotic to direct the immune response away from food allergy. In Three litters (12 pigs/litter) of Yorkshire pigs, piglets were randomly assigned to each of three treatment groups: control (PBS), vector (heat-killed *E. coli*), and treatment (heat-killed *E. coli* expressing IFN-γ). One-milliliter PBS or one ml heat-killed bacteria with/without IFN-γ were injected intramuscularly. The pigs were then allergized to Ovm by intraperitoneal injection together with cholera toxin as an adjuvant. To assess the reaction to Ovm pigs were fed egg white in yoghurt and assigned scores based on clinical signs of allergy during a two hour post-challenge observation. Clinical scores pooled by treatment groups across both litters indicated a significant difference in the number of pigs with clinical signs in the control group as compared to the vector and treatment groups (p < 0.0001). The vector and treatment groups did not differ significantly. Histamine concentrations in sera positively correlated to clinical scores (p = 0.0738; linear regression). The number of control pig sera that gave positive passive cutaneous anaphylaxis (PCA) was greater than for pigs in the vector and treatment groups (p = 0.0002 and p < 0.0001 respectively). There was no significant difference in the number of PCA positive pigs in the vector and in the treatment groups. It can be concluded that treatment of neonatal pigs with heat-killed *E. coli* significantly reduced subsequent frequency of allergy to Ovm. Inclusion of type 1
cytokine, IFN-γ had no additional effect on immune response. These results may reflect an induced type 1 immune response bias, possibly due to interactions of bacterial pathogen associated molecular patterns and pathogen recognition receptors on antigen-presenting cells. This may support aspects of the “Hygiene Hypothesis” for increased prevalence of allergy in western societies.

Evaluation of two washing machines on the bacterial load of dairy udder towels
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The objective of this study was to compare two different washing machines, the Maytag Neptune and the Miele, for their effects on the bacterial load of new cloth udder towels washed twice daily over a period of time. Little peer-reviewed information is available which documents a comparison of pre-milking management strategies, including type of towels used and the equipment and procedures for laundering them. Research was conducted at the Elora Dairy Research Center, which is milking approximately 140 Holstein cows twice daily. Two separate sets of udder towels where used for the duration of the trial. Five random samples were taken weekly from each machine prior to drying and placed in a freezer bag with of saline solution and agitated. Samples were cultured using 3M™ Peterfilm™ Plates for aerobic and coliform bacteria. Aerobic bacterial loads present in udder towels washed using the Miele were significantly lower than those washed using the Maytag throughout the trial. In addition, the aerobic bacterial load present in the towels washed using the Miele remained relatively constant throughout the trial, while there appeared to be a slow increase in the towels washed using the Maytag. There was no significant data to report on coliform bacteria from either machine. After eleven weeks a switch was made with respect to the machine in which the towels were washed. A sharp decline was observed in the red towels put into the Miele, and a similar sharp rise was seen in the blue towels washed in the Maytag. Future research should consider different test periods, comparing equipment of equal age, types of bedding used and types of udder towels.

Estrogen transport through the equine yolk sac wall
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In horses, about 16% of pregnancies diagnosed by ultrasonography at Day 15 (ovulation = Day 0) fail to result in a live foal. Most (60%) of these losses occur in the first month of pregnancy. The conceptus comprises the embryo proper, yolk-sac tissues that will form fetal membranes (and later the placenta), yolk-sac fluid, and an enveloping capsule. The yolk-sac wall has bilaminar and trilaminar components.

The concentrations of estrogens in the yolk sac fluid increase rapidly between approximately Days 12 and 24 and they are believed to be important signals in the interactions between the mare and her embryo. We hypothesized that estrogens egress more readily to target tissues (endometrium) through the thinner bilaminar yolk-sac wall than through the thicker trilaminar wall. Conceptuses (n = 6) were collected transcervically by uterine lavage between Days 15-18. Bilaminar and trilaminar tissues were separated under a dissecting microscope and their permeability determined separately using Valia-Chien Cells. Rates of transport through the bilaminar and trilaminar yolk-sac wall differed markedly, with tritiated estrone sulfate leaving the yolk sac more rapidly through the trilaminar than through the bilaminar tissue. Thus, our hypothesis was rejected. The difference may be due to active transporters present, perhaps in the mesoderm, in the trilaminar yolk-sac wall. Further work will be necessary to identify any such transporters, to extend the study to other steroid hormones, and to understand the relevance of differential transport to conceptus orientation and survival during the critical first month of pregnancy.

Characterization of antimicrobial drug use in Ontario sheep flocks and presence of antimicrobial resistance
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In recent years, studies have been undertaken to assess the prevalence of antimicrobial resistance (AMR) found in pathogens of agricultural animals and their products. To date, no studies have addressed this concern in commercial lamb production. The summer portion of this study was part of a larger prospective project that aims to assess drug use patterns, AMR and risk factors of AMR in the Ontario lamb industry. Fifty flocks were recruited and visited. At each visit, a survey assessing flock demographics, management practices and antimicrobial use was administered, followed by the collection of fecal samples. Samples were then cultured for Campylobacter spp., E.coli and Salmonella spp and each isolate tested for AMR. Of the samples tested to date, only one flock had one sample test positive for Salmonella enterica typhimurium; 3 flocks had samples test positive for Campylobacter jejuni; and all samples from the 6 flocks cultured for E.coli were positive. Preliminary results have revealed only two E.coli isolates that exhibited antimicrobial resistance. All other cultured bacterial isolates were susceptible to all tested antimicrobials. Questionnaire results suggest that antimicrobial drug use is common in the production of market lamb, with 90% of the flocks using some form of extra-label drug. Data and laboratory results

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will continue to be collected over the next twelve months and associations between antimicrobial use, management practices and AMR investigated. Results gathered at the end of the study period will provide insight into the role that management, flock size and drug use play in the development of AMR.

**Relationship between parameters used to estimate the burden of gastrointestinal nematode infections in sheep**

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Gastrointestinal nematodes are a significant problem in sheep farming and one species in particular, Haemonchus contortus, is capable of producing anemia. An on-farm parasite control system - FAMACHA® - has been developed to aid small ruminant farmers in identifying individual animals requiring treatment. This system has been used extensively in the southern USA and South Africa; however its use under Canadian conditions needs evaluation. The objective of this study was to determine the relationship between FAMACHA scores, fecal egg counts (FEC) and packed red cell volume (PCV) in sheep farms. In April/May 2007, 23 farms in Ontario and 11 in Quebec were visited; 10 ewes and 10 lambs were sampled on every farm for blood and feces, as well as body condition and FAMACHA scores. Blood and fecal samples were used to determine PCV and FEC, respectively. Significant Spearman rank correlations were found between PCV and FAMACHA in both ewes (-0.2809) and lambs (-0.4552). When agreement was measured between FAMACHA scores of 4 and 5 and a PCV threshold of less than 19%, both considered as anemic conditions, kappa was 0.386 (ewes) and -0.016 (lambs), with a sensitivity of 63.6% and 0%, and specificity of 95.3% and 99%, respectively. Based on these data the utility of FAMACHA® scoring can be questioned. However, further research in late-summer months is required to reach a concluding verdict about the method’s utility.

**Prevalence of Salmonella spp. and Clostridium difficile in the feces of horses housed at three different racetracks in Ontario**

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The objective of this study was to determine the prevalence of Salmonella spp. and Clostridium difficile in horse feces at 3 racetracks in Ontario. A fecal sample was collected from 200 different horses at 3 racetracks during the months of June and July. One hundred and fifteen Thoroughbreds and 85 Standardbreds were sampled. An enrichment process using CDNM broth, alcohol shock, and CDNM agar was implemented to isolate C. difficile via bacterial culture. Colonies were confirmed using colony morphology, smell and a positive l-proline aminopeptidase test. Salmonella spp. was to be isolated using MSRV agar where Salmonella has a specific migration pattern. Any positives would be plated on MacConkey for final isolation. Eight samples were positive for C. difficile, a prevalence of 4.0% overall. Zero samples were positive for Salmonella spp., a prevalence of 0%. Other studies determined prevalence of C. difficile ranging from 0% to 9.6%, but external factors or differences in methods may not have been accounted for between studies. Previous studies found prevalence of Salmonella spp. to be 0.6% and 0.8%. These numbers are similar to our prevalence but one should be cautious when concluding that there is no Salmonella spp. shedding occurring. Consecutive samples should be taken to be sure that the horse is not shedding Salmonella spp.

**Prevalence of Leptospira in wild raccoons at the Toronto Zoo**

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Although wildlife species, especially raccoons (Procyon lotor), are often reported to be potentially important natural reservoirs of *Leptospi r*sus various like *grippotyphosa* and *pomona*, no studies currently provide conclusive evidence that raccoons act as reservoirs. The purpose of this study was to conduct a preliminary assessment of the role of wild raccoons as potential reservoirs of *Leptospira* infections by: determining the seasonal pattern of seroprevalence of *Leptospira* in raccoons; and by, determining the prevalence of *Leptospira* shedding in urine and/or kidney samples from raccoons. From May to July 2007, 56 wild raccoons were captured in live traps on the premises of the Toronto Zoo (Scarborough, Ontario, Canada). Blood and urine samples were collected. Serum samples were tested for exposure to seven *Leptospira* serovars (*autumnalis*, *bratislava*, *canicola*, *grippotyphosa*, *icterohaemorrhagiae*, *hardjo* and *pomona*) using microscopic agglutination test. Urine and/or kidney samples were collected from 22 raccoons for culture and 16 of these were assessed for *Leptospira* bacteria by dark field microscopy at 1, 2, 4 and 6 weeks of culture. Only 1 of 63 samples collected from 56 raccoons captured had a marked serum antibody titre to *Leptospira* serovar *grippotyphosa* (titre 1:2560). All urine and/or kidney samples assessed were negative for *Leptospira* bacteria. With continued study through the fall, we may find seasonal differences in prevalence and provide better insight to the role of wild raccoons as potential reservoirs of *Leptospira* infection. Also, the role that other wildlife species, like skunks and...
Assessment of the ability of Holter recording indices to predict outcome in Dobermans with dilated cardiomyopathy
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It is unknown which indices on Holter recordings predict risk of sudden death (SD) or congestive heart failure (CHF) in dogs. The purpose of this study is to describe the frequency and character of ventricular premature contractions (VPCs) in asymptomatic Doberman pinschers, and assess the ability of Holter indices to predict risk of SD or CHF. This is a retrospective study of Holter data collected from 1999 to the present from 388 asymptomatic Dobermans. All recordings included in the study were limited to a minimum of 8 hours, totaling 374 recordings. Data collected included total number of VPCs, untriggered VPCs, couplets, triplets, salvos, episodes of ventricular tachycardia (VT), incidences of bigeminy, and number of VPCs displaying R on T. The numbers of the above events per hour were calculated by dividing the total for each variable by the Holter recording length. Outcome data was collected on 207 of these Dobermans and included date of onset of clinical signs (CHF or SD) and time from the last recording to onset of clinical signs or other non-cardiac outcome. Univariate and multivariate Cox proportional hazards analysis were used to identify Holter parameters associated with risk of onset of clinical signs (SD or CHF). Of the 374 dogs, 212 were female and 159 were male (3 of unknown gender). Median age was 5.8 years (range 0.5-13.6) and median body weight was 34.0 kg (range 22.7-57.5). The mean Holter length was 21.7 hours (range 8.2-26.2). Results of the univariate Cox proportional hazards analysis showed that age, body weight, VPCs/hr, couplets/hr, triplets/hr, presence of salvos, VT and bigeminy, and R on T/hr are associated with risk of onset of clinical signs of DCM. In multivariate proportional hazards models, when taking into account age and body weight, couplets/hr and presence of VT were significantly associated in several models with increased risk of onset of clinical signs.

The detection of female cell activity in male sex-chromosome chimeras using the Xist gene product
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The Xist gene is only widely expressed in females therefore; its gene product can be used as a marker for identifying normal females. Hence, detecting Xist gene expression in males (in tissues other than the testes, as Xist is expressed exclusively in the testes of males) may indicate that these males contain functional female cells and thus necessarily labels them as sex-chromosome chimeras. In the present study four previously identified male sex-chromosome chimeric sheep were screened for Xist gene expression and Xist cDNA was detected in three of the four chimeras. It is presumed that Xist cDNA was not detected in one of the chimeras because it contains a very small proportion of female cells in its blood and thus it is likely that the sample used did not contain female cells. None-the-less we conclude that the Xist gene product can be used as a marker for female cell activity.

Investigation of Canine Distemper Virus patterns in raccoons
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Raccoon submissions to the Canadian Cooperative Wildlife Health Centre (CCWHC) from Ontario were examined for the presence of spatial, temporal and spatio-temporal clusters of canine distemper (CD) submissions using spatial scan statistics. Based on Bernoulli models comparing CD to non-CD submissions, temporal clusters were identified in 2002 and 2006, and spatial clusters encompassing the Greater Toronto Area (GTA) and surrounding communities were identified in 2001 and 2005 when individual years were scanned. Using a space-time permutation model, spatio-temporal clusters were found in the years 2001, 2002, 2005, and 2006 surrounding the GTA and surrounding communities when the entire study period (2001-2006) was scanned. The temporal and spatio-temporal clusters were consistent with previously observed increases in CD among raccoons when the young disperse and when adults mate. Interpreting the biological significance of the spatial locations of the spatial and spatio-temporal clusters is complicated by the nature of these data and their impact on these statistical models. The models themselves should not be affected by purely spatial variation in raccoon populations or overall submissions, but regional variation in the types of diseases submitted for further investigation and the lack of submissions from certain regions of the province need to be considered when interpreting these results. However, the appropriateness of the CCWHC data for the study and surveillance of raccoon diseases around the GTA appears promising and is worth further investigation.

Statistical modeling of Standardbred race performance
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Traditionally, racelines have been used primarily to aid the betting public in attempting to predict race outcome. A
horse’s racelines describe the animal’s entire racing career, and contain information on pattern of performance in addition to events within a race. With increased focus on issues of poor or disappointing performance in the racing industry, tools are needed to increase the objectivity of clinical evaluations while supporting critical assessment of response to management change. Creation of multivariate models describing contributions of predictor information to outcome at each stage of a race is currently underway. This involves an iterative process of adding and subtracting terms, paying attention to the statistical significance of their contributions to the model and preserving hierarchy. The next objective in model creation will be to apply the models to data sets from horses that were not used in model construction to evaluate agreement between predicted and actual outcome, and therefore determine prediction intervals. The models can then be used to critically evaluate individual race and horse performance. Insights gained to date reveal some variables to be highly influential. Controlling for these will allow other variables with more subtle effects to be exposed. Construction of the models is made particularly challenging by the large number of variables (n=55), and the number of different levels within some variables (n>300). Objective and critical analysis of performance is possible for an animal with such a consistently detailed production record, and is limited only by the quality of the raw data.

Assessment of hepatic iron in Psittacine birds
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This study compares three methods of assessing hepatic iron concentrations in Psittacine liver samples. Hepatic iron was evaluated using a histological scoring system, microscopic image analysis, and measurement by atomic absorption spectrometry (AAS). Preliminary results demonstrate statistically significant correlations (p < 0.05) between histologic scores and iron concentrations measured by AAS (r = 0.64606) as well as between microscopic image analysis and AAS (r = 0.83652); histologic scores and image analysis are also significantly correlated (r = 0.90417). There were no significant correlations between measures of hepatic iron and species groupings, gender, age in months, or age category. Histologic scores, microscopic image analysis, and AAS measured on small biopsy samples were significantly correlated with the values derived from larger tissue samples (r = 0.94305, r = 0.95826, r = 0.91004). These results indicate that evaluating hepatic iron storage using a standardized scoring system on small endoscopic liver biopsies may be a useful indicator of hepatic iron concentrations in Psittacine birds.

Examination of sickness behaviour in dogs with lymphoma being treated with chemotherapy
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Chemotherapy has become a common treatment for dogs with cancer, offering a 60 to 90 percent remission rate for dogs with lymphoma. Unfortunately, chemotherapy can result in toxicities and alterations in behaviour such as fatigue, vomiting and anorexia. Although toxicity related to chemotherapeutics has been well documented, there has been little research focused on the behavioural changes experienced. This project investigated sickness behaviour in dogs with lymphoma treated with chemotherapy at the Veterinary Teaching Hospital, Ontario Veterinary College. The investigators utilized questionnaires, physical exams, video monitoring and Activity activity monitors (manufactured by MiniMitter Biotelemetry) to acquire information regarding activity level, anxiety, pain, appetite and side effects. At this time, there are preliminary results consisting of at least two weeks of treatment data for five of the dogs. Three of the five dogs have experienced vomiting at some point during their treatment. Of the dogs that presented with clinical signs, all of the owners have reported at least one week in which they saw improvement in their dog’s activity level. Knowledge in this area of cancer treatment will improve the welfare of dogs being treated by helping clinicians identify and alleviate any discomfort.

Investigation of systemic amyloidosis in Siamese and Balinese cats
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Systemic amyloidosis, a familial condition of Siamese and Balinese cats, is characterized by abnormal deposition of amyloid A protein in liver, spleen, kidney and other tissues leading to dysfunction of these organs and death of the animal in young adulthood. The objectives for this study were to describe the clinical and pathologic features of systemic amyloidosis, compare the sensitivity of staining methods, and investigate the molecular genetic basis of the disease. The study population was a single breeding group of 60 Siamese and Balinese cats with an ongoing problem of amyloidosis. Tissue samples were taken from all cats that died or were euthanized in the cattery in the past 4.5 years (n=35). Typical clinical signs of amyloidosis were lethargy, anorexia, weight loss, and many cats also had upper respiratory tract infections. Amyloid, identified in 77% of study animals, was detected most frequently in kidney, while the amount of amyloid was greatest in the spleen. The sensitivity and specificity of the H&E stain for detecting amyloid, compared to the Congo red stain, was 80.0% and 66.7% respectively. Congo red was a more accurate method
of identifying amyloid compared to H&E, and should be used for screening of tissues from all animals suspected of having or coming from genetic lines afflicted with the disease. Total mRNA was extracted from liver. Serum amyloid A (SAA) cDNA was amplified by RT-PCR and sequenced. A greater proportion of study animals had non-synonymous single nucleotide polymorphisms at positions 152 and 224 when compared to other control animals and other amino acid substitutions, suggesting that these polymorphisms contribute to development of amyloidosis in the Balinese and Siamese breeds.

The impact of acepromazine on the effectiveness of typical management practices for hypotension in dogs under deep isoflurane anesthesia
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Hypotension is a common anesthetic complication in veterinary medicine resulting from many factors, notably the anesthetic agents themselves. This study was designed to define the impact of acepromazine on the ease of producing hypotension during isoflurane anesthesia, and its effect on the cardiovascular response of three hypotension treatments, including an ephedrine infusion.

Once hypotensive (MAP<50 mmHg), six dogs were randomly assigned to either an acepromazine (A) or saline (B) pre-treatment followed by one of 3 treatments: dextran bolus(T1); crystalloid fluid bolus(T2); ephedrine bolus followed by a CRI (T3) in a latin square design. Cardiovascular measurements (heart rate, blood pressure (MAP), lithium dilution cardiac output (CO)) were taken at baseline, 5, 10, 15, 20, 30, and 40 minutes.

Group A required less isoflurane to achieve a mean arterial blood pressure (MAP) of 50 mmHg than group B (p=0.0036). The overall MAP response to treatment was affected by A or B pre-treatment (p<0.0001). T3 was most effective at increasing MAP in group B dogs. In group A, T3 significantly increased MAP at only 5 and 10 min compared to T1 and T2. In group B, T2 caused a reduction in MAP, while T1 maintained MAP. T3 improved CO compared to T1 and T2.

Acepromazine pre-medication made it easier to produce hypotension with isoflurane, however, treatments were less efficacious at improving MAP. Fluids did not improve MAP and initially worsened values. Dextran maintained MAP, but caused minimal improvement. A CRI of ephedrine maintained MAP compared to earlier work with a single bolus. Only ephedrine made major contributions to improving CO.

The effect of calf position at first feeding on the consumption of colostrum and success of passive transfer of immunoglobulins in dairy calves
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The objective was to evaluate the effect of calf position (either prior to or after the initial occurrence of standing) at the time of first feeding of colostrum on the quantity of voluntary consumption, and passive transfer of maternal immunoglobulins (Ig), as measured by serum total solids (TS) concentration. Newborn calves from 4 Holstein herds in central Ontario were categorized naturally into 1 of 2 observational cohort groups, based on position at the first offering of colostrum: (1) recumbent (R) or; (2) standing (S). The quantity of colostrum consumed by voluntary intake was recorded as well as timing relative to birth. Subsequent consumption of colostrum either by bottle or esophageal feeder, was recorded. Between days 1 to 5 of age, jugular blood samples were obtained and analyzed for serum TS concentration using digital refractometry. A total of 211 calves (110 R and 101 S) were enrolled. On average, R calves voluntarily consumed 0.16L less than S calves (1.67 L and 1.83 L, respectively) at the first feeding. R calves were 1.24 times more likely to have some level of voluntary consumption than S calves. Average serum TS was 5.29 g/dL and 5.32 g/dL for the R and S groups, respectively. Using the standard cut-off value of 5.2 g/dl, 87 (41.2%) of calves experienced failure of passive transfer. On average, calves received their first feeding of colostrum by 62 min of age. Results indicate that calf position does not affect quantity of voluntary colostrum consumption at the 1st offering and subsequent serum TS. Furthermore, ensuring a timely first feeding of colostrum helps but does not guarantee successful passive transfer of maternal Ig.

Prevalence and age-dependent occurrence of coccidia infections in pigs up to 8 weeks of age
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Isospora suis (coccidia) is a protozoan parasite that reproduces in the cells of the villi lining the small intestine. In the summer of 2006, research on 50 pig farms in southwestern Ontario showed that 70% of farms were positive for coccidia on the basis of testing piglets at 7 to 21 days of age. Furthermore, infections were associated with an increased risk of diarrhea and reduced growth rates (Webster et al. 2006).

In most countries, the only treatment available for coccidia infections in pigs is toltrazuril (Baycox). However, in 2005 the drug was banned in Canada, leaving pork producers without a drug with proven efficacy against coccidia. In order to maximize the chance of an alternative drug being
licensed for use against coccidia in Canadian pigs, information is required on the total impact of coccidia infections.

Since the 2006 Ontario study only sampled pigs up to 21 days of age, it may have underestimated the importance of coccidia. A study was therefore required to determine the impact of coccidia infections up to 8 weeks of age. In order to optimize the sampling design for this study, information was required on the shedding of coccidia up to 8 weeks of age. Up to now, shedding above 3 weeks of age has not been examined – it has been assumed that it does not occur.

A study was conducted at a farm previously known to have a coccidia problem in order to determine the optimal protocol for defining the coccidia-infection status of pigs up to 8 weeks of age. Rectal fecal samples were collected from randomly selected pigs representative of pigs from 1 to 8 weeks of age. When pigs were examined from 1 to 8 weeks of age for coccidia, 65% of all positive samples were detected at 15 to 24 days of age - the range in age that infected pigs were detected was 15 to 40 days. Above 24 days of age, low levels of oocyst shedding were typically detected. In conjunction with previous data, this study indicated that the optimal, practical, protocol for defining the coccidia-infection status of pigs up to 8 weeks of age is to examine fecal samples at weeks 2, 3 and 5 of life.

Effect of sucrose on perception of pain in rats
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There is an increasing focus on the use of non-pharmacologic approaches in the management and relief of pain in both human and veterinary medicine. In human medicine, several studies have shown that the administration of sucrose prior to a painful procedure in infants decreased pain sensitivity and increased sensitivity to pain-relieving medication. There have been few studies exploring these results in veterinary medicine. Initial research has been done on the effect of sucrose in infant rats with similar positive effects as per human infants, but that effect has not been explored in adult rats or in other species. This study examined the effect of oral sucrose on acute discomfort and physiologic stress in 32 male and 32 female Sprague-Dawley rats. The rats were pair-housed on a reversed light cycle in either a males-only room (n=16), a females-only room (n=16), or a mixed-gender room (n=32). Body weight and food consumption were measured weekly, and fecal samples were collected in the light and dark phases at 6 intervals during the study. Acute discomfort/pain was measured using the hotplate test, and physiological stress was measured by fecal corticosterone measurements using an ELISA assay. Body weight and food consumption rates were unaffected by gender housing (male/female vs mixed gender). There were no significant differences in hotplate latencies within gender regardless of housing paradigm; however, increased morphine latency was noted during Week 10 (saline latencies unaffected) in both genders, but was more marked in males. Previous studies in the lab have suggested that housing rats long-term in cages resulted in the blunting of Circadian cycling in fecal corticosterone levels, so a second objective of this study was to assess anhedonia in rats using two behaviour tests; a forced swim test (FST) and a novelty-seeking test. Results from this study are pending.

Proteomic analysis of normal and diseased canine cerebrospinal fluid
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Cerebrospinal fluid (CSF) is produced by the choroid plexus lining the ventricles and circulates throughout the central nervous system (CNS). CSF represents a unique proteome for neurological disease diagnosis. CSF has the most intimate contact with the CNS of any body fluid and contains proteins of nervous tissue origin that may be biomarkers for specific neurological diseases. Proteomics has become a refined technique in recent years since most disease states are reflected in protein concentration and structure changes. The purpose of this study was to generate a map of CSF proteins found in normal dogs and to identify disease-associated novel proteins. CSF samples from normal dogs and those with neurological diseases (categorized as intervertebral disc disease, abnormal mentation, granulomatous meningoencephalomyelitis or epilepsy) were separated by two-dimensional gel electrophoresis. Gel proteins were fluorescently stained and analyzed with Phoretix 2D software to compare spot volume and location between gels. Spots were excised from gels, digested, and identified by liquid chromatography followed by tandem mass spectrometry.

Results indicated that every CSF proteomic map from dogs with neurological disease differed from those of normal dogs. Five protein spots consistently increased by >2fold in 3 or more of the disease categories, while 5 other protein spots decreased by >2fold in 2 or more of the disease categories. Proteins changed in diseased dogs included specific inflammatory mediators and CNS metabolites. The identification of biomarkers could profoundly impact disease diagnosis and treatment, and further the understanding of neurological diseases in veterinary medicine.
Determining cytokine profiles in dogs with immune mediated diabetes: Phase 1 – Characterization of normal dogs
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The objective of this study was to establish a baseline for dog cytokine profiles using peripheral blood mononuclear cells (PBMC) cultured with antigens (roundworm, distemper virus, or Malassezia) or mitogens (ConA or pokeweed) using a sandwich ELISA. This information will be used as a basis for comparison to responses in diabetic dogs under similar circumstances. The hypothesis is that the majority of diabetic dogs are skewed towards a stronger TH1 response compared to normal dogs. Current results (6 TNF-α ELISA completed out of 30) show that the concentrations of TNF-α, the first cytokine examined, peak at 4 hours when PBMC are exposed to Malassezia. TNF-α concentrations also tend to increase with variation over time when PBMC are exposed to roundworm or distemper virus. Completion of this study will establish the Th1/Th2 cytokine parameters for comparison of normal and diabetic dogs.

Wild mammals as potential reservoirs of Salmonella and antimicrobial resistance in Southern Ontario
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Salmonellosis is the most common foodborne bacterial disease in the world. Food producing animals are the primary source of salmonellosis in humans; however, a Florida study found that 17% of apparently healthy wild raccoons (Procyon lotor) carried Salmonella serotypes also found in humans. Antimicrobial resistance (AMR) is a growing concern in human and veterinary medicine. Previous studies have shown conflicting results with regards to the prevalence of AMR in wild mammals. The purpose of this project was to determine the prevalence of Salmonella and Escherichia coli and the occurrence of antimicrobial resistance in the feces of selected wild mammals in Southern Ontario. Fecal samples were collected from 20 raccoons and 70 small mammals at the Toronto Zoo and at six sites within the Grand River water shed respectively. Three potentially zoonotic serotypes of Salmonella were isolated from 4 raccoons, suggesting that raccoons should be considered as potential reservoirs of Salmonella for humans and domestic animals in Southern Ontario. Antimicrobial resistant E. coli was isolated from the feces of 2 house mice and 10 raccoons. The most frequent resistances observed among the E. coli isolates in this study are the same as those found among isolates from food animals and humans. Further research is required to determine if wild mammals are important sources of infection for humans and domestic animals, and how they become colonized with these bacteria.

The effect of a Progesterone Releasing Intravaginal Device (PRID) on progesterone levels and pregnancy rates in previously inseminated dairy cattle
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A high metabolic rate in early lactation dairy cattle may result in the excessive metabolism of progesterone. This process may mean that the threshold levels required to maintain pregnancy are often not met; less than forty percent of fertilized follicles are maintained into early pregnancy. There is preliminary evidence to suggest that supplying exogenous progesterone increases pregnancy rates in early lactation dairy cattle. In this trial cows received either a progesterone releasing intravaginal device (PRID) or a placebo intravaginal device (PID) at five to eleven days following first insemination. Blood samples were taken at device insertion and removal to determine serum progesterone levels. Linear regression was used to control for progesterone concentration at insertion. Pregnancy diagnosis occurred at approximately three, five, and eight weeks following device insertion. Only cattle that retained their device for one week were included in the analysis.

The retention rates were 84.6% and 83.7% for PID and PRID, respectively. Within pregnant and open subgroups, treatment with PRID device significantly increased serum progesterone levels at removal relative to PID treated animals (P<0.05). Cows receiving the exogenous progesterone were 1.65 times more likely to become pregnant than cows receiving the placebo (P = 0.1).

The results of this trial suggest that the use of a progesterone releasing intravaginal device elevates progesterone levels such that early pregnancy is maintained, thus enhancing pregnancy rates in dairy cattle following first insemination.

Combining anti-angiogenic and cytotoxic therapies as an effective inhibitor of endothelial cell proliferation
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The formation of new blood vessels in an adult organism, termed angiogenesis, is an important process required not only for sustaining the health of the organism, but also in disease. Angiogenesis plays a major role in the pathology of cancer, and many studies have demonstrated that anti-angiogenic therapy may be useful in the treatment of this disease. Combination therapy, in which an anti-angiogenic drug is administered simultaneously with a cytotoxic drug,
has shown increased efficacy compared to monotherapies, but much work is still to be done. In this study, angiogenic inhibitors targeting the two different endothelial cell tyrosine kinase receptors were combined with a common cytotoxic chemotherapeutic drug cyclophosphamide (4-HC). Bovine aortic endothelial cells (BAECs) were treated with each drug both individually and together in order to determine the efficacy of combination therapy. This study shows that combination therapy is able to reduce endothelial cell survival and proliferation in comparison to each individual therapy alone.