Research highlights from the Ontario Veterinary College Summer Leadership and Research Program

This study was conducted under the supervision of various professors in the Ontario Veterinary College, University of Guelph.

The Summer Leadership & Research Program (SLRP) at the Ontario Veterinary College offers BSc, DVM and other summer student researchers at OVC a stimulating slate of events from May to August. The program includes a Round Table series with veterinarians and scientists working in diverse and intriguing areas, field trips to cutting-edge research facilities and the Metro Toronto Zoo, and professional development workshops to prepare for conference-style sessions.

MARKERS OF MALIGNANCY IN CANINE OSTEOSARCOMA

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Osteosarcoma is the most common primary bone tumor in dogs comprising approximately 80% of all primary bone neoplasms. It occurs most commonly in the metaphyses of long bones and is characterized by aggressive local invasion and early pulmonary metastasis. There is a lack of good prognostic indicators for this cancer in dogs. Mitotic index has been used to assess proliferation rate in a variety of cancers and is often used to help grade tumours. As well, recent work using a mouse model of osteosarcoma has implicated the type 1 alpha regulatory subunit of cAMP-dependent protein kinase (PRKAR1A) in osteosarcoma tumorigenesis. Loss of PRKAR1A is associated with a favorable response to chemotherapy in human osteosarcoma. The first objective was to determine if mitotic index differs between high and low PRKAR1A expressing canine osteosarcoma tumours from Veterinary Teaching Hospital (VTH) over the past ten years. The second objective was to compare post-chemotherapy survival of canine osteosarcoma patients with high versus low PRKAR1A expressing tumours. It was found that PRKAR1A expression does not correlate with mitotic index, but post-chemotherapy survival was significantly longer in patients with PRKAR1A low expressing tumours. This implies that the survival advantage afforded by low PRKAR1A expression is not related to differences in mitotic index between high and low expressing tumours. Although there are low numbers of cases with low PRKAR1A expressing osteosarcomas, these patients have significantly longer survival times. More cases will be needed to confirm the usefulness of PRKAR1A immunostaining as a prognostic indicator in canine osteosarcoma.

TRANSCRIPTION FACTOR AND CYTOKINE GENE EXPRESSION IN BLOOD MONONUCLEAR CELLS OF DAIRY CATTLE CLASSIFIED ON THEIR ABILITIES TO MOUNT ANTIBODY OR CELL-MEDIATED IMMUNE RESPONSES

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A patented protocol has been developed at the University of Guelph for phenotyping dairy cattle based on their abilities to mount antibody (AMIR) and cell mediated (CMIR) immune responses. Animals identified as high responders for both traits exhibit decreased disease incidence and enhanced responses to vaccines while maintaining important production traits. In an effort to better understand the gene expression profiles underlying these phenotypes, the expression of key genes involved in the initiation and maintenance of effective AMIR and CMIR were measured. Transcription factor TBX21 and cytokine IFNG are central regulators of CMIR; while the transcription factor GATA3 and cytokine IL4 regulate AMIR. To investigate the role of these transcription factors and cytokines in biased IR phenotypes, heifers classified as highAMIR/lowCMIR (n=12) and lowAMIR/highCMIR (n=12) were identified. Venous blood was collected and mononuclear cells were isolated and cultured under different conditions: culture media, media + ConA and media + ConA + antigen (C.albicans, Hen Egg-White Lysozyme). Cells were
harvested at 4, 16, 24, 48, 72 and 96 hours, RNA isolated and TBX21, IFNG, GATA3 and IL4 mRNA expression quantified using RT-qPCR. Although experiments are ongoing, we hypothesize that the biased highAMIR/lowCMIR and lowAMIR/highCMIR phenotypes will be associated with a bias in the expression of key transcription factor and cytokine genes. Specifically, highAMIR responders are expected to express enhanced levels of GATA3 and IL4, while highCMIR responders are expected to express enhanced levels of TBX21 and IFNG. The results of this study will contribute to a better understanding of the basis of variation in immune response phenotypes and have implications for improving dairy cattle health.

**EPIDEMIOLOGICAL SURVEY OF AVIAN BORNAVIRUS IN BIRDS IN ONTARIO**

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Proventricular dilation disease (PDD) is a disease of psittacine species (parrots) which primarily affects the autonomic and central nervous systems, resulting in gastrointestinal malfunction, wasting, and neurological signs. Histologically, characteristic lymphocyte infiltrations can be seen within nerves and ganglia associated with the gastrointestinal tract, as well as within the brain and spinal cord. A novel avian bornavirus (ABV) has been discovered in tissues of psittacine birds affected with PDD, and recent research has supported it as the cause of the disease. Researchers at the University of Guelph have recently identified ABV in tissues from free-ranging Canada geese and trumpeter swans. A retrospective study was designed to determine the prevalence of avian bornavirus in wild waterfowl with clinical or pathologic lesions similar to those seen with PDD. Wild waterfowl cases were selected from the Toronto Zoo (TZ) and the Canadian Cooperative Wildlife Health Centre (CCWHC) databases from 1992 to date, utilizing selection criteria based on history, clinical signs and necropsy findings. Suspect cases were examined histologically and with immunohistochemistry (IHC), and fresh/frozen tissue was submitted for reverse transcriptase polymerase chain reaction (RT-PCR) for the ABV nucleoprotein gene. Eleven of 12 geese and 2/2 trumpeter swans tested by IHC were positive, and 6 of the positive geese were also PCR positive. The bornavirus nucleoprotein gene recovered from these 6 Canada geese showed 100% homology on nucleotide and protein levels; however, its sequence differed from that found in psittacines and other avian species. Preliminary results indicate that ABV should be considered a differential diagnosis for wild waterfowl presenting with neurological signs and GI malfunction/impaction. Susceptibility and implications for other species remain to be determined.

**THROMBOELASTOGRAPHY IN DOGS FOLLOWING ACUTE TRAUMA**

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Coagulation abnormalities occur in human trauma patients and are predictive of increased mortality. Previous studies using traditional coagulation tests show that alterations in platelet numbers, clotting factors, and clotting inhibitors also occur in dogs following trauma. Thromboelastography (TEG\(^{®}\)), a global measurement of clot formation and strength, will detect coagulopathies in dogs following trauma and abnormalities will be associated with increased mortality. Eighteen client-owned dogs sustaining trauma were enrolled with owner consent. Blood samples were collected on days 1, 3, and 5 of hospitalization for kaolin-activated TEG\(^{®}\) analysis. Clinical and laboratory variables including outcome were also recorded. When comparing dogs following acute trauma to healthy dogs, R was significantly decreased (p<0.04) within 12 hours of the traumatic episode (day 1). On days 3 and 5, the angle, MA, and CI were significantly increased and K was significantly decreased (p<0.05). Over the course of hospitalization, R, MA, and CI significantly increased, while K decreased (p<0.05). Increased lactate concentration correlated with an increased K (p=0.002). There was no association between TEG\(^{®}\) parameters and survival. Within 12 hours of trauma, dogs exhibit a shorter time to initiation of clot formation compared to normal dogs. By day 3, time to initiation of clot formation is normal; however, the speed of clot formation and clot strength are increased. This suggests that dogs are hypercoagulable following trauma and that lactic acidosis may be associated with the development of coagulopathies.
THE PREVALENCE OF ANTHelmINTIC-RESISTANT GASTRO-INTESTINAL NEMATODES IN ONTARIO SHEEP FLOCKS

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Anthelmintic-resistant gastro-intestinal nematodes threaten the sustainability of sheep farming worldwide. The objective of this study is to determine the prevalence of ivermectin drench failure on Ontario sheep farms, and the proportion associated with anthelmintic resistance. Twenty farms were enrolled in May and June, 2010. From the beginning of the grazing season, fecal samples were collected monthly from 15 randomly selected sheep in their first grazing season. When the mean fecal egg count (FEC) exceeded 200 eggs per gram (epg) of feces, producers treated the group with ivermectin drench; drench checks were performed by comparing pre-treatment FECs with FECs 14 days after treatment. On farms with drench failure (i.e. <90% mean FEC reduction), a Fecal Egg Count Reduction Test (FECRT) was performed in which research team members treated animals with ivermectin; an untreated control group was also included. Farms with <95% reduction in mean FEC are considered to have ivermectin resistance.

As of September 1, 2010, ivermectin drench failure has been documented on 12 of 16 (75%) farms tested, with 4 farms still having to be tested. These data suggest that ivermectin drench failure is a common problem in Ontario sheep flocks. The FECRTs that are currently being carried out on the farms with drench failure will determine the proportion of drench failure associated with ivermectin resistance and the proportion associated with inaccurate treatment. This information will allow veterinarians and producers to take appropriate actions to reduce the prevalence and spread of anthelmintic resistance in sheep.

PRODUCER PERCEPTIONS OF DISEASES ASSOCIATED WITH SHEEP AND SHEEP PRODUCTS

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A survey of all dairy and 450 randomly selected meat sheep producers in Ontario was conducted to investigate producers’ perceptions on zoonotic and foodborne diseases in their industries. Focus groups conducted with producers prior to survey design served to inform the content and vocabulary of the questionnaire. The questionnaire included questions on the producer’s confidence level in their knowledge of foodborne and zoonotic diseases, and in their physician’s knowledge of these diseases, as well as their preferred method of knowledge translation and transfer (KTT).

Response rates were 29.3% and 32.4% for the meat and dairy producers, respectively. Approximately 46% of dairy and 42% of meat producers were ‘somewhat’ or ‘not at all’ confident in their knowledge of foodborne diseases, and 58% of dairy and 74% of meat producers were ‘somewhat’ or ‘not at all’ confident in their knowledge of zoonotic diseases. The majority of producers thought it was important that they receive more information on these diseases. The top three methods of KTT preferred by producers were: a producer handbook, industry publications and summaries sent via regular mail. Approximately, 66% of dairy and 84% of meat producers said it was important that their physician receives educational information on these diseases. The majority of producers from both commodity groups showed support and desire for further research on foodborne and zoonotic diseases including: Johne’s disease, listeriosis, cryptosporidiosis, chlamydial abortion, orf and salmonellosis. These results will be used to direct future research projects that will be of direct relevance to Ontario sheep producers.

KEEPING YOUR BIRDS HEALTHY – BIOSECURITY BASICS FOR SMALL FLOCKS

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Recent infectious disease outbreaks in North America have caused serious financial catastrophes, industry shutdowns, and widespread flock eradictions in both the commercial and non-commercial poultry industries. Because of this, food safety, human health and on-farm biosecurity have become growing concerns. The University of Guelph, Poultry Industry Council, and Ontario Ministry of Agriculture, Food, and Rural Affairs have worked together to develop a biosecurity education initiative targeting the non-regulated feather industry, who have limited access to on-farm biosecurity basics. After researching the many non-regulated feather groups such as game birds, pheasants, waterfowl and
fancy birds, the researchers found that these groups needed more information relating to disease defence and a network for communication. As well, these groups needed proper healthcare from their local veterinarians. This initiative has developed informative resources in the form of a biosecurity kit including disease, anatomy and management fact sheets, logbooks, and posters; a comprehensive website at www.healthybirds.ca; and a successful workshop specializing in avian pathology for non-poultry veterinarians to meet these needs. Currently, the initiative is being promoted with the goal of educating every non-regulated feather producer in Ontario. With a province-wide, industry wide focus on on-farm biosecurity, the hope is that disease can be drastically decreased from the commercial and non-commercial avian industries.

**IMPROVED END OF LAY HEN WELFARE DURING TRANSPORTATION VIA TRAILER ENHANCEMENT**

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1Little Rock Farm Trucking*
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This project aims to improve the current welfare concern with end of lay hen transportation through the development of a comfortable, humane method of transportation from grower to processor. Overall handling of spent hens during the catching and hauling process will be reduced via pullet carts and an enhanced transport trailer will be created to ensure a viable bird upon arrival at the processor during all seasons. This project will provide an improved method of transport that will better comply with an advanced industry and its’ animal welfare concerns.

Trailer enhancements will include: adjustable ventilation slits, built in fans to improve air circulation, a solid roof to provide shade and a moisture free environment as well as in trailer temperature and humidity monitors. During testing, the new prototype trailer will be loaded alongside the current trailer model. Birds throughout the load will be randomly selected for temperature monitoring as well as glucose collection before and after transportation. Results will then be analyzed to determine any significant differences between the trailer types.

The development of this system, before welfare concerns expand further within society, will show a pro-active rather than a reactive response from the Ontario poultry industry. This type of response will show the general consumer that the welfare of food animals is of concern and is taken seriously within the agriculture industry. Meeting the current demand for an improved method of transport, will show the advancement of the industry and may entice a change nation and continent wide.

**WELFARE CONSIDERATIONS FOR SHEEP UNDERGOING THE RECTO-ANAL MUCOSAL ASSOCIATED LYMPHOID TISSUE (RAMALT) BIOPSY**

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The recto-anal mucosal associated lymphoid tissue (RAMALT) biopsy is used to detect pre-clinical scrapie infection in sheep. There is a need to determine the best method to mitigate pain sheep experience during and after the RAMALT biopsy. The purpose of this study was therefore to determine if the procedure is painful as well as look at 3 different pain mitigation techniques. This was done by observing behavior during the biopsy as well as looking for behavioral differences pre- to post-biopsy between treatment groups. Groups of 10 sheep were treated with ketoprofen, locally-applied lidocaine gel, both or nothing(control group). There were no statistically significant differences in behavior between treatment groups or from pre to post biopsy. Sheep in the treated groups may have behaved the same as sheep in the control group because: 1. the procedure may not have resulted in sufficient post-operative pain to disturb their general behaviour, 2. because all treatments may have been ineffective at reducing pain, 3. the sample size may have been too small. In order to address some of these issues a second trial is to be performed to help increase the sample size.

**GOAT PRODUCER PERCEPTIONS OF FOOD-BORNE AND ZOONOTIC DISEASES**

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Goat production in Ontario is expanding, with an increase in demand for goat meat, milk, cheese and other dairy products. 1. But with this expansion comes the challenge of producing safe products for humans, while also maintaining healthy animals and their caregivers. Researchers at the University of Guelph would like to move forward with new research on food-borne and zoonotic diseases in the goat sector, and include producer input to make sure the research is relevant and effective.

Focus groups and questionnaires were completed by producers to determine how goat producers perceive...
zoonotic and food-borne diseases in their animal production, directions for new research, and effective ways to communicate that research to producers. It was found that producers are confident in their knowledge of food-borne and zoonotic diseases of goats but also wish for more information. Information in a printed producer handbook would be well received. Producers would support research projects on Q-fever and Johne’s Disease.

Outcomes of this research include the following: all participating producers were mailed summaries of this research, articles on zoonotic diseases are being published in producer publications, a sero-survey of Q-fever infections in small ruminant herds and herd workers, and a project on Johne’s disease in dairy small ruminants have been started, and future small ruminant research projects conducted by the authors will emphasize the participatory research approach and knowledge translation and transfer activities (KTT). KTT methods will involve those identified as being most effective.

**ANTIMICROBIAL RESISTANT ESCHERICHIA COLI IN WILDLIFE TRAPPED ON LIVESTOCK FARMS**

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Antimicrobial resistance (AMR) is a global concern for animal, human, and environmental health. Antimicrobial resistance is typically thought to develop as a consequence of antimicrobial use; however, AMR has been found in wild animals with no known direct exposure to antibiotics. We are testing the hypothesis that livestock farms act as a source of AMR for wild animals. Previous work has shown that AMR is more common in wild animals living on swine farms compared to natural areas and the objective of this study is to determine if the prevalence and patterns of AMR in wildlife varies with farm type and reflects what is seen in production animals. Up to three E. coli isolates from wild animals (mice, voles, shrews, raccoons and groundhogs) and production animals on swine, beef, and dairy farms in southern Ontario were tested for resistance to 15 antimicrobials. This is an ongoing study and to date we have results for samples collected from wild mammals on 4 swine, 2 beef, and 2 dairy farms. Resistance to >1 antimicrobials was detected in 12 of 96 (13%) isolates. Resistant isolates were detected in wildlife from all farm types; however, the frequency of resistance varied with farm type (5% on beef farms, 12% on swine farms, and 27% on dairy farms). The results from production animals are pending. Although further work is needed these preliminary results suggest that the prevalence of AMR in wildlife may vary depending on farm type.

**CHEMOSENSITIVITY OF CANINE OSTEOSARCOMA CELL LINES**

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Osteosarcoma is the most common primary bone malignancy in canines and the current standard of care involves amputation followed by adjuvant chemotherapy. Although long term survival is generally poor, tumours respond differentially to chemotherapy which is not yet understood. The purpose of this study was to characterize canine osteosarcoma cell lines based on their doubling time and correlate this finding to their chemosensitivity. Doubling time was determined by plating 18 dishes of equal numbers of cells for each cell line and counting their increase in cell number every 2 to 3 days. A logarithmic graph was created to determine the doubling time of each cell line. The same cell lines were seeded at a cell concentration of 10,000 and 20,000 cells per well and then treated with doxorubicin at various doses (0-800 ng/mL). After an incubation period of 72 hours, cell viability was measured using an MTT assay. The most rapidly dividing cell line was D17 with the highest chemosensitivity (IC50= 97 ng/mL). In contrast, the second fastest cell line was JL 13 with the lowest chemosensitivity (IC50= 231 ng/mL). Our results indicate that there is no correlation between doubling time and chemosensitivity of these cell lines (r²= 0, p= 0.99). This suggests that proliferation rate is not a major predictor of chemosensitivity in these cell lines.

**ESTABLISHING THROMBOELASTOGRAPHY REFERENCE INTERVALS FOR CATS USING THREE METHODS: CITRATED NATIVE, KAOLIN ACTIVATION AND TISSUE FACTOR ACTIVATION**

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Thromboelastography (TEG) is a test of hemostasis that is widely used in human medicine. Recent veterinary studies have reported the use of TEG mainly in dogs. Using TEG in cats represents a novel and informative opportunity for assessment of feline hemostasis: To evaluate feline hemostasis using TEG with three methods; citrated native, kaolin activation and tissue factor activation, as well as to assess the variability between the two samples taken per feline. Jugular venipuncture was conducted in 20 cats, with citrated blood collected for TEG analysis. TEG analysis was
performed on citrated native, kaolin-activated, and tissue factor-activated blood. Two hours later, this procedure was repeated from the opposite jugular vein, yielding a total of 120 analyses. Four parameters were recorded from each tracing; Reaction time (R), alpha angle (α), kappa (K) value, and maximum amplitude (MA). Tissue factor activation resulted in the most variable alpha angles, while kaolin activation led to the most variable R times. Significant differences were found between methods in regard to R time and alpha angle, but not for MA. No significant differences were found between the first and second tracings. Reference intervals for R times were 2.63-7.49, 2.02-5.43 and 0.29-0.91 minutes, for CN, K and TF activation, respectively; while intervals for alpha angle were 47.37-70.46 (CN), 46.58-83.63 (K), 52.49-88.26 degrees (TF). MA reference intervals were from 33.58-61.29 (CN), 33.44-66.28 (K) and 27.82-61.59 mm (TF). For cats with kappa values, intervals were 0.35-4.74 (CN), 0.11-3.78 (K) and 1.22-5.81 (TF). The three methods of TEG analysis used in this study appear appropriate for use in healthy cats. There were significant differences between the three methods with respect to R times and alpha angle, thus methods cannot be used interchangeably. Now that reference intervals have been determined using healthy cats, these can now be used as a baseline to compare cats with coagulopathies and other disorders of hemoestasis.

**Sow Lameness**

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Sow lameness is a leading cause for culling sows of any age. In this study the objective was to find the prevalence of lameness in gestating sows in a commercial herd. Each sow is scored based on gait and given a score of 0, 1, or 2, 0 being sound, 2 obviously lame. A video outlining the scoring system was watched by the researchers prior to scoring and each sow was scored by two people. However, this is a subjective process so in this study an infrared camera was used to take images of the sows scored manually. Infrared imaging is expected to be much less subjective as a means of determining sow lameness. The infrared imaging portion of this study is ongoing. Of 564 sows were manually scored at farm #1, 4.4% received a score of 1. Housing on this farm was a mixture of pens and individual stalls with lameness prevalence of 2.6% and 4.7% for pens and stalls respectively. No sows on farm 1 scored a 2. On a second farm 207 sows were scored with an overall lameness of 5.6%. This farm also used a mixture of housing types. On this farm 2.9% of the sows housed in pens scored a 2 and 7.7% scored a 1. Only 0.6% of the sows housed in stalls scored a 2 and 1.8% scored a 1. Stacking density for loose-housed sows was lower on farm 2. There was no obvious reason for the higher lameness prevalence in the second herd. This small pilot study illustrates the difficulty of measuring lameness and determining its causes.

**Breeding Protocol at Arkell Swine: A Case Study**

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The retrospective research investigated the breeding protocol at Arkell Swine Research Station over the time period of January 1<sup>st</sup>, 2009 to December 31<sup>st</sup>, 2009 to determine how age at first breeding and backfat thickness at time of selection affected farrowing performance and culling rates among the herd. A total of 204 breedings were analyzed based on age at first breeding and backfat thickness readings adjusted to a body weight of 100kg. Records were retrieved from from Herdsman™ Swine Records and the Canadian Centre for Swine Improvement. The majority of gilts selected for breeding had backfat thicknesses between 10 and 16 mm. There was no correlation between backfat composition and culling rates or performance. Gilt age, in contrast, appeared to affect overall performance, with the greatest performance being achieved when gilts were within the age of 240-280 days of age when first bred. Average weaned piglet values for gilts aged <240 days, 240-280 days, and >280 days are 8.3, 9.0, and 8.2 piglets, respectively. The results show gilts less than 240 days of age, as well as gilts aged older than 280 days, showed a decrease in piglets born, piglets weaned, and also show an increase in culling rates of 6 and 18%, respectively. Upon evaluation, it can be concluded that delaying breeding at Arkell Swine until the age of 240 days can increase overall productivity of the herd, but maintaining gilts in the herd beyond 280 days is both costly and associated with reduced performance.