Equine Guelph
Research Update

Spring 2016

helping horses for life

Lights, Camera, Digestion

The successful capture of video footage of the inside of the horses' digestive tract has earned Equine Guelph staff member Diane Gibbard her Masters degree. Gibbard defended her thesis investigating capsule endoscopy (CE) as an imaging tool for horses on August 19, 2015. Congratulations go out to Diane and her advisor, Dr. Jeff Thomason of the Ontario Veterinary College, whose research produced high quality video footage capturing healthy intestinal villi and exposing common parasites and lesions in various parts of the small intestine.

Congratulations to Diane Gibbard who successfully defended her thesis investigating capsule endoscopy as an imaging tool for horses.

In the last ten years, CE has emerged on the scene as a more patient-friendly procedure in human medicine to help diagnose intestinal disorders by detecting bleeding, lesions, inflammation and tumors in the small intestine.

Traditional endoscopy procedures, via mouth or rectum, are incapable of reaching and assessing the small intestine. CE works by swallowing a small camera encased in a capsule (complete with a light, transmitter and battery to power it). The "pill" then travels down the esophagus, through the stomach and four to six metres of small intestine while taking pictures. This procedure can be done without anaesthetic and allows people to carry on with their day while wearing sensors and a recording device to collect the data.

The prospect of using CE to investigate equine gastro-intestinal (GIT) problems has both great appeal and challenges. Like humans, horses suffer from a variety of GIT disorders and being able to access sections of the GIT for examination is even more challenging given the much longer length of the intestinal tract (24-30m) and larger abdomen.

Potential CE applications include diagnostics for recurring colic and malabsorption syndrome which can both present with similar symptoms including weight loss.

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Welcome to the Equine Guelph annual report on the ground-breaking research funded by partners, sponsors and donors to Equine Guelph's Research program. This 13th anniversary issue celebrates the small and mighty innovations in equine research, including a camera the size of a pill for exploring the horse's digestive system.

Be sure to follow links to the expanded article on exciting developments in stem cell therapy and an accompanying video.

We hope the research updates that benefit both the racing and non-racing sectors give you an informative overview of the current status of research being funded with the help of the industry partners of Equine Guelph.

Dr. Jeff Thomason, Co-Chair
Equine Guelph Research Committee

UNIVERSITY of GUELPH

CHANGING LIVES IMPROVING LIFE

INSIDE **Volume 13**

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Current diagnostic tools such as ultrasound and x-ray technology have limitations in reaching the small intestine as the equine abdomen is quite wide, encased in a thick body wall and ribcage. Another alternative, exploratory surgery comes with risk and great expense.

"Tethered endoscopes do not have the reach to examine a large portion of a horse's small intestine," says Gibbard. *"Capsule Endoscopy has the potential to become a useful imaging tool for the small intestine and could help in diagnosing causes of intestinal disorders in cases of colic and malabsorption syndrome, leading to more targeted treatment."*

The first challenge begins with safely emptying the horse's GI tract of food while maintaining hydration and motility. It is easy to tell a human patient to fast and drink eight litres of water. Horses, on the other hand, "you can lead them to water but can't make them drink!" In light of this, water boluses were used in addition to free choice water to help keep horses hydrated and maintain gut motility.

Horses were placed on a reducing feeding ration prior to a 12-hour fast and were administered an anti-ulcer medication. The capsule was delivered nasogastrically into the stomachs of two Standardbred horses over four sequential trials, recording for twelve hours each trial.

Stay tuned for the release of video captured using capsule endoscopic technology revealing tapeworms, pinworms and lesions in the horses GI tract.

The short clips of high quality unique footage captured clearly point to the potential for CE as an imaging tool for the future, but image obstruction from residual feed proved to be challenging. Capsule signal strength was a factor, with horses possessing far more mass than humans. Higher image transmission was noted in the smaller of the two horses suggesting better transmission results when there is less tissue and body fat through which to travel.

Before the fourth trial, the capsule was modified enabling twice the signal strength, which improved image transmission overall, but the variance between transmissions from the smaller to larger horse remained. Placement of the electrodes to receive transmissions and other tracking challenges will also require further investigation to allow for accurate analysis of the capsule position at all times and to help solve issues of intermittent reception. "Take two" to move this very new capsule endoscopic technology forward will require further research.

Funding for this project was provided through Equine Guelph, Mr. Claude Margue (private donor), OVC Dean's Fellowship Scholarship with significant in-kind support from Halton Equine Veterinary Services and Intromedic Inc.

Story by – Jackie Bellamy-Zions

2016 – Year of Biosecurity

Spread the Word
not the germs

Welfare continues to be top of mind in Equine Guelph's communications. "Spread the word, not the germs" will be the biosecurity mantra for 2016 monthly *e*-News features; preparing horse owners for the new National Farm-Level Biosecurity standard for the Equine Sector. The standard will be the first document of its kind, providing a detailed scientific approach to biosecurity, making it a good reference tool. A producers' guide will also be published giving horse owners useful "how to" information. Both aim to help reduce the risk of disease transmission, minimize the impact of outbreaks and improve the health and welfare of horses.

Biosecurity and the implications of the new standard were discussed in an interview with Dr. Alison Moore, Lead Veterinarian Animal Health and Welfare at the Ontario Ministry of Agriculture, Food and Rural Affairs. Moore joined the development committee for the National Farm-Level Biosecurity standard in the winter of 2014. The committee was comprised of equine owners, subject matter experts, provincial government officials, representatives from regional and sector-specific equine industry organizations, the public sector and academics; including representatives from Equine Guelph. The standard was developed through a partnership of Equine Canada and the Canadian Food Inspection Agency (CFIA), in collaboration with Agriculture and Agri-Food Canada (AAFC).

In food animals, the importance of biosecurity standards are recognized in economic trade and for protecting animal health. *"The equine sector is unique,"* says Moore. *"It is exciting to be a contributor to this new standard for the horse industry which will serve as a guideline for equines at the individual farm level right up to international competition."*

There are misconceptions that biosecurity is not important to the small stable owner or keepers of closed herds. Another myth is vaccination will provide an inclusive disease prevention plan. *"Others fear complicated protocols, with images of people in Hazmat suits conjured up when you utter the word biosecurity,"* says Moore.

Key points from the voluntary standard and producers guide will be discussed in Equine Guelph's monthly *e*-News to *spread the word* and help protect horses from getting sick. Sign up at EquineGuelph.ca

The reality is biosecurity is more about diligence than difficulty. Moore explains, *"Maintain doing the small things until they become part of the routine."* Protecting your horses from disease involves very simple practices such as: handwashing, not sharing equipment between horses, being cognizant of where you graze at shows and not patting unfamiliar horses and then returning to work with your own.

Vaccination is an important part of every facility's biosecurity plan but should never be the only measure taken. Having a plan in writing is an important step to make sure all topics of biosecurity are considered and addressed.

The plan should include: access, traffic control, separating new arrivals, pasture management and disinfection routines.

"Communication is key," emphasizes Moore, *"Every barn's risk for disease is different but every member of the barn community needs to be familiar with and able to communicate the biosecurity plan clearly."* This includes: the training of staff, expectations from service providers, visitors and boarders. Optimizing health on the farm is the responsibility of all.

Story by – Jackie Bellamy-Zions

Biosecurity Barn Poster for free download at: *http://www.equineguelph.ca/pdf/facts/EquineBiosecurityPoster_%20Oct2015.pdf*

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Good Things in Small Packages - Stem Cell Therapy

"It's approximately four millimeters in diameter," exclaimed Ontario Veterinary College researcher, Thomas Koch, unable to contain his excitement. The tiny disk of equine cartilage, manufactured in the OVC lab, is full of potential.

A cartilage injury can mean the end of an athlete's career. Damaged joint cartilage does not repair on its own and often leads to early osteo-arthritis. Great progress has been made, in Koch's lab, by PhD student Sarah Lepage, in collaboration with Dr. Rita Kandel from the University of Toronto.

They are putting together a protocol for making tissue engineered cartilage constructs. The next step will be evaluating them in live horses. *"We are standing on the shoulders of research pioneered by Dr. Mark Hurtig and Kandel,"* says Koch, crediting the developers of the mosaic grafting technique of bone and cartilage for sheep and horses. The graft keeps transplanted tissue in place for the healing process to begin.

Now that the equine cartilage disk is a reality, the question becomes "how will it hold up to the larger forces exerted in a horse?" There is further refinement and testing to be done before the first live trial on an equine, potentially by 2017. This topic is sure to be of great interest to human medicine as well, given the similar cartilage thickness and great athletic ability of the horse.

Koch says, *"It is very exciting and satisfying to be this close to testing the tissue construct in a horse."* Ten years of exploratory work, finding the best possible cells, carrier substances and protocols are paving the way to revolutionary methods of treating cartilage damage with cell-based therapies.

The Best Cells

Mesenchymal Stromal Cells (MSC's) are the building blocks of bone, cartilage, fat, muscle and tendon. When Koch began isolating MSC's in 2005 he discovered they could be sourced from umbilical cord blood of newly born foals.

His team discovered isolating MSC's from jugular blood was also possible but not as reliable. Cord blood cells taken at birth are younger and possess superior capabilities of dividing and creating different types of tissues. Koch explains, *"They are easy to make into cartilage cells and better at it than other alternatives such as using cells from bone marrow, adipose tissue and equine-induced pluripotent stem cell (iPSC)."*

Promising Results

Injection of cord blood MSC's into joints of research horses has proven safe in multiple studies.

In collaboration with Dr. Judith Koenig, Clinical Studies at OVC, former PhD Candidate, Dr. Lynn Williams, made significant contributions to research, performing a live study injecting lipopolysaccharide (LPS), which induces a temporary inflammatory response. He performed injections with and without MSC's. In the blinded study, results showed less inflammation in the equine joints for the injections which included MSC's.

Williams also studied how injected stem cells suppress lymphocytes, a modulatory function of the immune system. In a comparison study, he

The equine cartilage disk is now a reality with live testing on the horizon.

Mesenchymal Stromal Cells collected from umbilical cord blood when a foal is born are the cell of choice for Koch's research, *"They are easy to make into cartilage cells and better at it than other alternatives. They are also strong modulators of the immune system"*

Watch video at: equineguelph.ca/research

Updates


Photo by: Dr. Judith Koenig

No adverse reactions have been noted in three client-owned horses with tendon damage following cord blood MSC injection and all three horses returned to work.

A stem cell is a cell that can copy itself, divides extensively and can diversify into a new tissue (such as cartilage).

When Koch began isolating Mesenchymal Stromal Cells (MSC's) in 2005 he discovered they also exist in umbilical cord blood of newly born foals.


Photo by: Ritesh Briah

"It is exciting working with a really good team of students making significant contributions," says Dr. Koch, photographed with PhD candidate Sarah Lepage.

discovered there is no difference between using frozen cells (freshly thawed out of liquid nitrogen), and those that have been thawed and allowed to adjust for a week in the lab. The clinical applications of having viable cells for treatment right after thawing from liquid nitrogen eliminates the need for a cell culture facility and expedites treatment.

Williams also played a role in determining the best carrier solution to transport the MSC for clinical use in collaboration with a U.S. company, BioLife Solutions. The commercial carrier solution, free from biologics, is now used when testing MSC in live horses. *"Often stem cells are combined with other biologics, but the absence of biologics in this formula allows our researchers to attribute the results of stem cell treatments directly to MSC's,"* emphasizes Koch.

More success stories were recorded in the last few years when three client-owned horses with tendon damage returned back to work, following cord blood MSC injection. No adverse reactions were noted and all three were treated with MSC cord blood acquired from another horse. In the one horse, saline was used as the carrier and in the other two, the BioLife HypoThermosol was used. This work was also done in collaboration with Dr. Judith Koenig.

One case was a breeding stallion, with such a severe tendon injury, he was unable to mount the phantom for collection. He became a candidate for stem cell therapy after previous surgery and extensive rehabilitation proved unsuccessful. He returned to work four months later, after two MSC cord blood injections, given one month apart. The imaging specialist was very surprised when the ultrasound revealed incredibly fast healing of the tissue including fiber alignment and filling of the defect. Drs. Koch and Koenig are now recruiting horses with tendon injuries for a controlled study to determine efficacy of the stem cells in tendon healing.

Koch explains, "*The effect seen in the tendons after MSC stem cell therapy is most likely due to the cells immunomodulatory properties,"* meaning they are capable of modifying or regulating one or more immune functions. The MSC cells are:

1) influencing and reducing inflammation at the site
2) providing a micro-environment that is more conducive to healing
3) secreting different factors that are influencing the immune system

Bright Future, More Live Studies on the Horizon

"We are interested in exploring how these cells are able to modulate the immune system," says Koch, *"as well as evaluating MSC's for their capacity to become different cell types, and in particular cartilage."* Investigating the secretory functions is a future topic of study for the OVC lab, with a PhD student ready to assist. Koch would like to investigate the possibilities of isolating secretions for use and learn how they can modulate the immune system.

With an excellent team behind him, Koch says, *"With so much leg work completed, we are looking forward to exciting times in the future with more in-vivo work."*

Dr. Thomas Koch is an assistant professor in the Department of Biomedical Sciences at the Ontario Veterinary College and an adjunct associate professor in the Orthopedic Research Lab at Aarhus University in Denmark. His work is funded by the Danish Research Agency for Technology, Production and Innovation, Grayson Research Foundation of Lexington, Kentucky, BioE Inc. of Minnesota, USA, SentrX Animal Care Inc.of Utah, USA, and Morris Animal Foundation (USA), the Canadian Foundation of Innovation – Leaders Opportunity Fund, Pet Trust and the Equine Guelph Research Fund. A federal grant from Natural Sciences and Engineering Research Council of Canada (NSERC) was instrumental in attaining graduate student stipends from the Dean's office. Studies performed at Arkell research station in collaboration with BioLife Solutions were made possible with funding from the Ontario Ministry of Agriculture Food and Rural Affairs. OMAFRA has been a key partnership allowing in vivo studies to proceed at manageable costs.

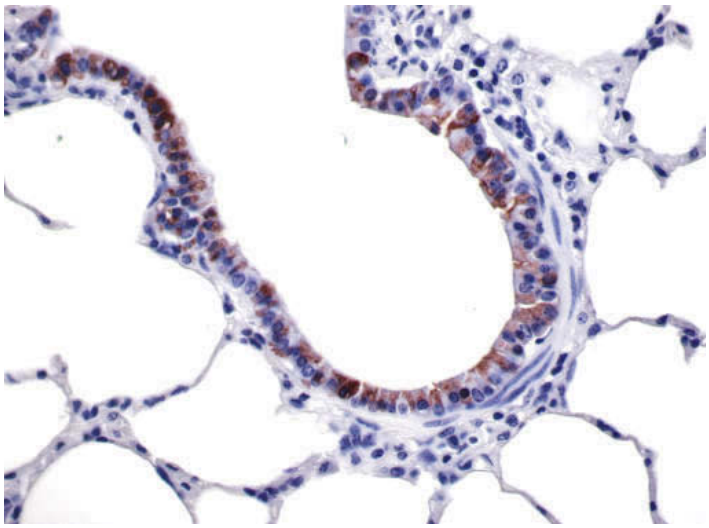
Story by – Jackie Bellamy-Zions

Full length article online at http://www.equineguelph.ca/news/index.php?content=469

Deep in the Data Mine for Heaves Research

"By the time symptoms of heaves are noticed; the irreversible damage is done," explains Ontario Veterinary College researcher, Dorothee Bienzle. Years of exposure to dust, bacteria and mould takes its toll on the equine respiratory system. Horses diagnosed with heaves do not tend to return to high performance work.

Bienzle has been studying the global lung epithelial response to inhaled dust from tissue samples collected in 2013 from horses with and without heaves. The exciting part of the research is discovering how the lung responds to injury. The epithelium is normally a very specialized tissue, with multiple cells with different jobs. Some produce mucus, others have cilia to move particles, some produce Clara Cell Secretory Protein (CCSP), some surfactant, and so on. After repeated exposure to dust and mould, the epithelial cells start to lose their specialized functions and production of proteins is impacted. Bienzle says, *"The change in the epithelium is very dramatic."* Once damaged, it cannot effectively combat future challenges.



Lung section immunohisotchemically stained for CCSP 3

Photo by Dr. Dorothee Bienzle

Intensive data mining and software analysis is being used to unravel the roles specific genes are playing and how they relate to one another as the body attempts to reduce inflammation. Much of the genome mapping of the horse remains unknown and Bienzle's doctoral student Laurence Tessier spends a great deal of time using software to cross reference findings with the human genome.

In the next step of research, Bienzle and Tessier will be performing more data mining to look at gene variants. More testing will be done on the samples procured in collaboration with Dr. Laurent Viel through bronchoalveolar lavage, pulmonary function testing and biopsy. Using immunohistochemistry Bienzle will be confirming that key genes they have identified from tissue biopsies thus far are producing the proteins they have predicted. By putting an antibody to the protein on the biopsy slides they will be able to localize and verify which protein is present.

Preventing Heaves

1) Rest and Recover after illness

2) Steer clear of round bales

3) Feed top quality (be vigilant choosing hay free of mould, especially if it has been a wet growing season)

Funding to date for this research has been provided by Equine Guelph, Canada Research Chairs (CRC) program, Natural Sciences and Engineering Research Council of Canada (NSERC) and Ontario Ministry of Agriculture and Rural Affairs. An Ontario Veterinary College fellowship has also been awarded for Laurence Tessier.

Story by – Jackie Bellamy-Zions

A Decade of EquiMania! Fun at the Royal!

EquiMania! is celebrating its 10th Anniversary as a featured education centre at the Royal Agricultural Winter Fair, November 4 - 13, 2016. Equine Guelph's award-winning attraction is a fun and interactive youth exhibit promoting horse health and safety.

"We are passionate about getting kids and adults excited about horses," says Gayle Ecker, director of Equine Guelph. *"Thanks to the generosity and vision of our loyal partners, we are able to celebrate a wonderful milestone at the Royal, continuing in our mission of leveraging the EquiMania! program to educate and introduce youth to the equine industry."*

Back again for 2016, the interactive EquiMania! Explorer safety initiative takes youth through a series of stations where they learn how to be safe around horses, in the stable, around equipment and in the barn yard. This year's spotlighted safety topic will be fire prevention and safety on the farm.

"This is a partnership that has made sense for over 10 years," says Dean Anderson, executive director of Workplace Safety & Prevention Services. *"We are delivering a critical safety message in a fun, interactive way to kids and if it prevents one accident or saves one life, it is a wise investment."*

Story by – Henrietta Coole

How Does Equine Assisted Therapy Affect Horses?

Do horses respond differently to people with post-traumatic stress disorder (PTSD)? Prof. Katrina Merkies, Animal Biosciences at the University of Guelph plans to find out. She has received a $10,000 innovation research grant from the Horses and Humans Research Foundation, which supports research on equine-assisted therapies and will begin a study this summer.

"The effect of equine assisted therapy on humans has received increasing amounts of study but very little is known about the impact on the horse," says Merkies.

For the study, four adult volunteers with PTSD will spend time in an enclosure with a horse, all the while being videotaped. Then, after viewing the footage, four adult actors will enter the enclosure, each imitating one of the PTSD patients. Neither will have physical contact with the horses but may talk, yell and move about as they choose.

Horse behaviour will be observed such as: gait, head height, body orientation, ear orientation and distance to human. The horses will be wearing heart rate monitors and saliva samples will be taken. Data gathered will compare heart rate and cortisol concentrations to measure stress.

The study will occur at Sunrise Therapeutic Riding and Learning Centre in Puslinch, Ontario, collaborating with program director and head instructor, Nikki Duffield. The 20 horses to be used have all gone through a strict selection process, used by Sunrise, including a trial period to determine the horse's suitability for their program.

"*It will be interesting to see if the length of time horses have spent in the program has an effect on their response to humans,"* says Merkies. *"I am also very interested in the role of personality - both horse and human - in the horse-human relationship. Are there specific personalities of horses that mesh better with specific personalities of humans to achieve the most benefit from equine assisted therapy?"*

Photo by: Helen Macgregor

Dr. Katrina Merkies and her past Grand Prix dressage horse Schooner

To ensure participant safety, two instructors (as well as the researchers) will be present at all times during the research trials. All instructors have been trained and certified with the Canadian Therapeutic Riding Association (CanTRA). If you are interested in participating in this research ethics board approved study, please contact kmerkies@uoguelph.ca.

"The use of horses in therapy is growing," says Merkies, *"so for their well-being, it's important to determine the behavioural and physiological impacts of this therapy on the horses themselves."*

Story by – Jackie Bellamy-Zions

Photo by: Sharon Grose

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EVENTS

Mark your calendar!

Can-Am All Breed Equine Expo (Markham)– EquiMania!
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Equine Guelph's Online Courses
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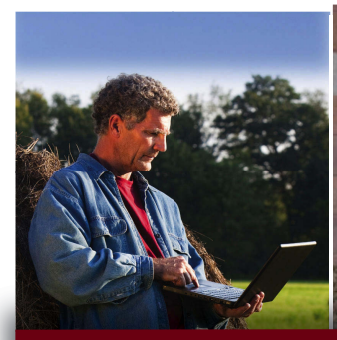
Anyone wishing to excerpt Equine Guelph should contact: Jackie Bellamy-Zions ext 54756 jbellamy@uoguelph.ca



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