The 2013 Annual Report covering the period of January 1, 2013 through December 31, 2013 is enclosed.

For this reporting period, The Harry M. Zweig Memorial Fund for Equine Research Committee granted approval of 7 of 14 submitted projects. Five were new studies, and two were revised. Three continuation awards were also approved. The total amount allocated for 2013 awards was $471,394. Copies of the investigators’ reports are provided.

Additionally, Cornell Hosted its fifth annual poster session celebrating the collaboration between the Harry M. Zweig Memorial Fund for Equine Research and Cornell University. Cornell’s faculty, students, and staff showcased their research to the research community and the Harry M. Zweig Memorial Fund for Equine Research Committee at the annual poster session on Wednesday, November 13, 2013.

### 2013 Harry M. Zweig Memorial Fund for Equine Research Awards

<table>
<thead>
<tr>
<th>CONTINUATION AWARDS</th>
<th>AWARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lisa Fortier</td>
<td>$50,302</td>
</tr>
<tr>
<td>Identification of the Optimal Biologic to Enhance Endogenous Stem Cell Recruitment and Homing for Facilitated Musculoskeletal Tissue Regeneration (Year 2)</td>
<td></td>
</tr>
<tr>
<td>Tracy Stokol</td>
<td>$69,003</td>
</tr>
<tr>
<td>The Role of Platelets in the Pathogenesis of Equid Herpes Virus Type-1 Infection (Year 2)</td>
<td></td>
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<tr>
<td>Bettina Wagner</td>
<td>$68,875</td>
</tr>
<tr>
<td>A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates (Year 2)</td>
<td></td>
</tr>
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</table>
## NEW AWARDS

<table>
<thead>
<tr>
<th>Name</th>
<th>Project Description</th>
<th>Funding (Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorothy Ainsworth</td>
<td>Fine Mapping of Candidate Genes Contributing to equine Left Recurrent Laryngeal Neuroptathy (RLN) (1 year award)</td>
<td>$45,828</td>
</tr>
<tr>
<td>Douglas Antczak</td>
<td>T-Cell Mediated Immunity and Vaccine Development in Horses (2 Year award)</td>
<td>$50,000</td>
</tr>
<tr>
<td>Lisa Fortier</td>
<td>Cellular Biomarkers of Early Cartilage injury Measured with Multiphoton Imaging (1 year award)</td>
<td>$46,412</td>
</tr>
<tr>
<td>Thomas Divers</td>
<td>Etiology and Prevention of Equine Serum Hepatitis (Theiler’s Disease) (2 year award)</td>
<td>$67,000</td>
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<tr>
<td>Norman Ducharme</td>
<td>An exploratory Study into the Practical application of a Regenerative Medicine Approach to Reconstruction of the Equine Upper Airway (1 year award)</td>
<td>$96,977</td>
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<tr>
<td>Alan Nixon</td>
<td>Osteoarthritis Control through Combinatorial Stem Cell Therapy (1 year award)</td>
<td>$80,147</td>
</tr>
<tr>
<td>Bettina Wagner</td>
<td>Innate Immune Mechanisms and T-Cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses (2 year award)</td>
<td>$85,030</td>
</tr>
</tbody>
</table>

### Interim & Completed 2012 Awards

Dr. Lisa Fortier’s project entitled “Identification of the Optimal Biologic to Enhance Endogenous Stem Cell Recruitment and Homing for Facilitated Musculoskeletal Tissue Regeneration.” Dr. Fortier received a no cost extension through June 30, 2014. A progress report is included herein.

Dr. Linda Mittel’s project entitled “Detection of Spirochetes, Rickettsia, and other Bacteria and Parasitic Protozoa (often vector born) that Cause Fevers of Unknown Origin in Horses and in Horse-Associated Ticks in the Northeast, Mid-Atlantic, and Great Lakes Regions” received a no cost extension through June 30, 2014. A final report will be included next year.

Dr. Tracy Stokol’s project entitled “The Role of Platelets in the Pathogenesis of Equid Herpes Virus Type-1 Infection.” Dr. Stokol received an additional no cost extension through June 30, 2014. A progress report is included herein.
The Incentive Program enables the Fund to leverage its investment in Zweig-sponsored research by encouraging Veterinary College faculty to seek either additional or supplementary monies from external sponsors that base their award decisions on a process that involves informed scientific review. The external grant must be closely related to a Zweig project. Eligible sponsors include, but are not limited to, the Grayson Foundation, the NIH, the NSF, and the USDA’s National Research Initiative. Recipients provide an annual report on the use of these funds.

The following external grant awards resulted from Zweig funding:

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>External Award</th>
<th>Sponsor</th>
<th>Project Period</th>
<th>Awarded Amount</th>
<th>Incentive Award</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Lisa Fortier</td>
<td>Cellular Biomarkers of Early Cartilage injury Measured with Multiphoton Imaging</td>
<td>Weill-NIH-CTSC</td>
<td>1/1/13-12/31/14</td>
<td>$54,457</td>
<td>$2,723</td>
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<tr>
<td>Kira Novakofski</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr. Doug Antczak</td>
<td>Genetic Studies of Equine Sarcoid Tumors</td>
<td>Morris Animal Foundation</td>
<td>01/01/13 - 12/31/14</td>
<td>$46,296</td>
<td>$4,629</td>
</tr>
<tr>
<td>Samantha Brooks</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

PUBLICATIONS

Publications resulting from awards from the Harry M. Zweig Memorial Fund for Equine Research during 2013 were:

Increasing Platelet Concentrations in Leukocyte-Reduced Platelet-Rich Plasma
Boswell, SG, Schnabel LV, Mohammed, HO, Sundman EA, Minas T, Fortier LA.

The anti-inflammatory and Inflammatory and matrix Restorative Mechanisms of Platelet-Rich Plasma in Osteoarthritis
Sundman EA, Cole BJ, Karas V, Della Valle CJ, Tetreault MW, Fortier LA.

Preovulatory Progestagen Treatment in Mares Fails to Delay Ovulation
Canisso IF, Gallacher K, Gilbert MA, Korn A, Schweizer CM, Bedford-Guaus SJ, Gilbert RO
Effects of Clopidogrel on Horses with Experimentally Induced Endotoxemia

Antibodies to OspC, OspF and C6 antigens as Indicators for Infection with Borrelia Burgdorferi in Horses. Wagner B, Goodman LB, Rollins A, Freer HS

Genomic Analysis Establishes Correlation between Growth and Laryngeal Ceupopathy in Thoroughbreds

Identification of Cartilage Injury using Quantative Multiphoton Microscopy
Novakofski KD, Williams RM, Fortier, LA, Mohammed HO, Zipfel WR, Bonassar LJ

Identification of the Optimal Biologic to Enhance Endogenous Stem Cell Recruitment
Homes H, Wilson B, Silverberg J, Goerger J, Fortier L

Development of an Equine Model of Talocrural Post-Traumatic Osteoarthritis
Delco M, Bonassar L, kennedy K, Tuan R, alexander P, Foriter L

Response of Cartilage Impact Between Multiple Joints and Implications for the Development of PostTraumatic Osteoarthritis

Chondrocyte Death Quantified using FDA-Approved Fluorescein is Increased Near Matrix Cracks Resulting from Traumatic Injury
Novakofski K, Williams R, Bonassar L, Fortier L

Genomic Analysis Establishes Correlation between Growth and Laryngeal Neuropathy in Thoroughbreds
Boyko AR, Brooks SA, Behan AL, Castelhano M, Corey E, Oliveira KC, Todhunter RJ, Zhang Z, Ainsworth DM, Robinson NE


Genetics of Recurrent Laryngeal Neuropathy

Brooks SA
The 5th World Equine Airways Symposium, July 16, 2013, Calgary, Canada (invited presentation).

Effects of Hypoglossal Nerve Block and Electrical Stimulation of the Thyrohyoideus Muscles on Position of the Larynx and Hyoid Apparatus in Healthy Horses.

Zantingh AJ, Ducharme NG, Mitchell LM, Cheetham J

Effects of Clopidogrel on the Platelet Activation Response in Horses


Jonathan Cheetham, VetMB, PhD, DipACVS
Zweig Principal Research Scientist 2012-2013

At the November 15, 2012 Annual meeting, Dr. Jonathan Cheetham was appointed as the first Zweig Research Scientist 2012-2013. His appointment is for the period January 1, 2013 through December 1, 2013, with a possible renewal to be approved at the next annual meeting of the Zweig Committee. Acknowledgement of this prestigious award is included herein.
Dr. Cheetham is honored to have been named the first Harry M. Zweig Memorial Fund for Equine Research Principal Research Scientist, noting that the award is an exceptional recognition and honor for an early career investigator. He appreciates the award, and honored that the Zweig Committee and College recognized his efforts as a clinician scientist and provided the award to foster his research. The award has had a tremendous impact on the contributions made on behalf of the Harry M. Zweig Memorial Fund for Equine Research at the College of Veterinary Medicine at Cornell University, and has allowed him to pursue and focus on a line of investigation and development that will lead to regenerative therapies for equine laryngeal disease. The research is progressing well, and the ongoing, long-term project will produce a fundamental shift in the way in which horses are treated for Recurrent Laryngeal Neuropathy (RLN), or “Roaring” which is a significant performance limiting problem in both racehorses and sports horses.

Career Development Activities

During the past fiscal year, he also received a promotion from Research Scientist to Principal Research Scientist. Additionally, his laboratory was successful in obtaining a three-year NIH “Small Grant Award” and he was honored as the recipient of the 2013 Zoetis Research Excellence Award in Animal Health. Career development activities have included, attending a Young Investigators grant writing workshop held by the United States Bone and Joint Initiative and serving as co-chair of the Department of Clinical Sciences Research Committee.

This ongoing work will have widespread application and would not have been possible without support from the Harry M. Zweig Fund for Equine Research. He is grateful for the support and the protected time to pursue research to advance the field, and this prestigious award has made a profound impact on his career.
CORNELL CLINICAL FELLOW IN EQUINE HEALTH

At the 2007 Annual meeting, the Harry M. Zweig Committee approved the allocation of funds to help support a Cornell Clinical Fellow in Equine Health. Dr. Sophy Jesty was selected as Cornell’s first Clinical Fellow, followed by Dr. Sarah Pownder, and more recently another individual has been identified as a Clinical Fellow, and supported in part by Zweig funds, and all have been highly successful. Cornell’s College of Veterinary Medicine’s two-year Clinical Fellows Program is the first in the country to address a growing shortage of academic veterinarians who conduct research on animal diseases and basic biology. The program is designed to help students meet the financial and time demands of qualifying for a position in veterinary academic medicine, which has traditionally required students to complete an M.S. or Ph.D. after they finish their doctorate in veterinary medicine (DVM). The two-year program, available to veterinarians who have completed a three-year residency, offers an annual salary of $65,000 plus benefits and an additional $15,000 per year to fund a research project.

OUTREACH 2013

Patent updates for 2013

During 2012, Dr. Chang applied for patent 3080-10 “Novel Immunologenic Proteins of Leptospira” patent application number 13/459,791

In 2013 patent D-3080-07 – “Immunogenic Proteins of Leptospira” US patent 8,168,207, was issued to Dr. Chang on May 1, 2013.

Provisional patent application No. 61/903,619 (US); submitted 11/13/2013; “Stimulation of Neonatal Immunity”. Inventors: B. Wagner, G. Perkins; Applicant: College of Veterinary Medicine, Cornell University, Ithaca, NY (2013).

Zweig News Capsules

There were two issues of the Zweig News Capsule published in 2013. Copies of these issues can be found in Appendix (E).

All Zweig News Capsules can be found at the Zweig Website at: and the latest one is attached: http://www.vet.cornell.edu/zweig/

SUMMARY OF EXPENDITURES

The 2013 Summary of Allocations was presented and approved at the Zweig Committee Annual Meeting in November 2012 (Appendix B).

2014 ZWEIG PROGRAM

Six projects were approved for funding, from a roster of 12 applications, at the Harry M. Zweig Memorial Fund annual November 2013 meeting. The list of projects funded for 2014 are shown in (Appendix D).
<table>
<thead>
<tr>
<th>Dr. Ainsworth</th>
<th>Fine Mapping of Candidate Genes contributing to Equine Left Recurrent Laryngeal Neuropathy (RLN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Antczak</td>
<td>T-Cell Mediated Immunity and Vaccine Development in Horses</td>
</tr>
<tr>
<td>Dr. Divers</td>
<td>Etiology and Prevention of Equine Serum Hepatitis (Theiler’s Disease)</td>
</tr>
<tr>
<td>Dr. Ducharme</td>
<td>An Exploratory Study into the Practical Application of Regenerative Medicine Approach to Reconstruction of the Equine Upper Airway”</td>
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<td>Dr. Wagner</td>
<td>Innate Immune Mechanisms and T-Cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses</td>
</tr>
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</table>
2013 Progress report

<table>
<thead>
<tr>
<th>P.I.</th>
<th>Dr. Dorothy Ainsworth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>Fine Mapping of Candidate Genes Contributing to Equine Left Recurrent Laryngeal Neuropathy (RLN)</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/11-12/31/13</td>
</tr>
<tr>
<td>Reporting Period:</td>
<td>1/1/13-6/30/13</td>
</tr>
</tbody>
</table>

Dr. Ainsworth was granted a no cost extension through June 30, 2014. A Progress report is attached.
PROJECT TITLE: Fine Mapping of Candidate Genes Contributing to Equine Left Recurrent Laryngeal Neuropathy (RLN)

PRINCIPAL INVESTIGATOR(S): DM Ainsworth, NE Robinson, J Swinburne, RJ Todhunter, Z Zhang, J Stick, NG Ducharme.

A. GWAS of 550 Thoroughbred horses establishes a correlation between withers height and RLN. In horses affected with RLN, the distal axon of the recurrent laryngeal nerve, the longest nerve in the horse’s body, degenerates causing secondary paresis to complete paralysis of the intrinsic laryngeal muscles. Neurogenic atrophy of one particular laryngeal abductor, the left cricoarytenoideus dorsalis (CAD), prevents maximal abduction of the left arytenoid cartilage. This results in laryngeal airflow obstruction, poor pulmonary gas exchange and reduced athletic performance. The prevalence of RLN ranges from 2-11% in Thoroughbreds and 35-46% in Draft horse breeds (Lane et al., 1987; Brakenhoff et al., 2006). The presence of genetic factors contributing to RLN is suggested by the observation that offspring of RLN-affected stallions are more likely to be affected with the disorder than are offspring from unaffected stallions (Ohnesorge et al., 1993). However, prior to our team undertaking this study in 2011, the genetics of RLN had not been extensively investigated in Thoroughbreds.

Using a cohort of 550 well-phenotyped Thoroughbred horses, we conducted a GWAS using the newer Illumina Equine SNP70 Beadchip (65, 157 markers). We first performed a GWAS on withers height using sex and gelding as covariates (heights were recorded for 505 Thoroughbreds) and found strong signal association on ECA3 (104.4-109.0 Mb). The strongest association signal (P = 1.3 x 10^-22) was centered on SNP BIEC2_808543 (Figure 3A) adjacent to the ligand dependent nuclear receptor corepressor-like (LCORL) and non-SMC condensing I complex subunit G (NCAPG) genes. Our results confirmed independent studies documenting a strong association between the LCORL/NCAPG region and body size in the horse (Marvandi-Nejad et al, 2012; Tetens et al, 2013). In our study we found that although height differed significantly between mares, geldings and stallions, gender and gelding only explained 7% of the variation in height in the samples. In contrast the LCORL/NCAPG minor (G) allele count at BIEC2_808543 had an effect of increasing height 3.7 cm (SE ± 0.4) and explained 16.7% of the variation in height in the data set. This effect was roughly additive and consistent in sires, mares and geldings (Table 1, below right).

![Figure 3: The Manhattan Plots (sex and gelding as covariates). Table 1: Possessing](image)

<table>
<thead>
<tr>
<th>N</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
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</thead>
<tbody>
<tr>
<td>Stallion</td>
<td>79</td>
<td>41</td>
<td>6</td>
</tr>
<tr>
<td>Gelding</td>
<td>157</td>
<td>68</td>
<td>5</td>
</tr>
<tr>
<td>Mare</td>
<td>137</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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<td>173</td>
<td>19</td>
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<table>
<thead>
<tr>
<th>Average Height (cm)</th>
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<tbody>
<tr>
<td>Stallion</td>
</tr>
<tr>
<td>Gelding</td>
</tr>
<tr>
<td>Mare</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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</table>

<table>
<thead>
<tr>
<th>Risk of RLN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stallion</td>
</tr>
<tr>
<td>Gelding</td>
</tr>
<tr>
<td>Mare</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>
Possessing the G allele at the BIEC2_808543 SNP demonstrate the association of height with ECA (Panel A) and of is associated with a greater height and risk of having RLN. RLN with ECA3 (Panel B). [From Boyko et al., 2013] [From Boyko et al., 2013]

A GWAS for RLN (sex and gelding as covariates) demonstrated the strongest association with RLN (Panel B Figure 3) near the same LCORL/NCAPG locus (ECA3 105.1-105.5 Mb). Including height as an additional covariate in the model yielded no more significant associations although regions of ECA23 (36.2-39.8Mb) and ECA18 (65.8-67.0 Mb) showed suggestive associations with RLN (P = 5.6 x 10^-6 and 1.6 x 10^-5, respectively; See Boyko manuscript in Appendix for these Manhattan plots). GWAS for RLN without covariates also yielded an additional suggestive association on chr X at 57.8 Mb as shown in Figure 1 (page 2). Thus 4 QTLs were associated with RLN, the strongest signal being on ECA3.

Next, linkage disequilibrium among the SNPs was investigated for the height and RLN locus on ECA3. A haplotype (i.e. a set of SNPs on a single chromosome that are associated statistically) for height consisted of an eight SNP block spanning ECA3 (105.3-105.8 Mb, P = 8.18 x 10^-23), a genomic region that included the LCORL/NCAPG genes (Figure 4, below). A haplotype generating the smallest P-value for RLN (P = 6.29 x 10^-11) consisted of a seven SNP block (105.8-105.9 Mb) which overlapped with the height haplotype block by two markers and also included the 5' half of the FAM184B gene.

Figure 4: Shown are the statistical and spatial relationships between SNPs (vertically labeled by name) in the candidate region for height and RLN. The haplotype block for height is highlighted in black (triangle) and that for RLN is in shown in gold. Haplotype location relative to ECA3 genes is also shown.

B. A custom-designed, high density SNP panel improves resolution of ECA3 locus (Focus of 2013 Zweig). In an effort to separate RLN SNPs from their close association with the height SNPs, we designed a high density SNP panel consisting of 150 new and 53 previously tested SNPs. The new panel improved coverage of ECA3 (115 SNPs) as well as that of ECA18 (25 SNPs) and chr X (27 SNPs). We also included SNPs on ECA6 (31 SNPs), ECA9 (3 SNPs) and ECA 11 (2 SNPs) because these chromosomes contain genes associated with equine growth and size (HMGA2; ZFAT, LASP1). The Thoroughbred DNA set was again genotyped, the data were re-analyzed and new Manhattan Plots were generated. Shown in Figure 5 is the new GWAS for RLN (with sex and gelding as covariates). Green dots represent SNP P-values from the custom-designed panel; black and gray dots are those from the EqSNP70 chip. Note that additional SNP P-values exceeded Bonferroni significance showing that we had indeed improved our coverage of ECA3. No SNPs on ECA18 or chromosome X exceeded threshold for significance. (See Figure 2, page 3 for RLN Manhattan Plot without covariates). The new haplotype analysis is shown in Figure 6, below. Note that the RLN haplotype block has moved further to the right of the height haplotype block but the two blocks remain associated, reflecting the long LD of the
Thoroughbreds. Thus, to further interrogate these regions, we propose to conduct an across-breed approach by studying RLN in Belgian Draft Horses. These horses are ideal because they have much shorter linkage disequilibrium and a higher prevalence of RLN compared to the Thoroughbred. At the end of our studies we hope to dissociate the RLN locus from the height region, or conclusively prove that they are one in the same, and identify candidate genes responsible for RLN.

Figure 5: RLN GWAS – ECA3 signal
Figure 6: Revised haplotype plot based upon data from custom-designed SNP panel is enhanced. (Custom) and EqSNP70 (GWAS). RLN locus (gold) is located further to the right of, but remains associated with the height locus due to the long LD in Thoroughbreds.
Harry M. Zweig Memorial Fund  
for Equine Research

2013 Annual Report

<table>
<thead>
<tr>
<th>P.I.</th>
<th>Dr. Douglas Antczak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>T-Cell Mediated Immunity and Vaccine Development in Horses</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/13-12/31/14</td>
</tr>
<tr>
<td>Reporting Period:</td>
<td>1/1/13-12/31/13</td>
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</table>
PROJECT TITLE: T-cell Mediated Immunity and Vaccine Development in Horses

PRINCIPAL INVESTIGATOR(S): Douglas F. Antczak, Rebecca Tallmadge, Nikolaus Osterrieder

Original Specific Aims: These aims have not been modified.
1) To identify the most important MHC class I molecule that presents immunogenic peptide after EHV-1 infection in a common and well-studied horse MHC type;
2) To determine the amino acid sequence of the EHV-1 peptide bound by that MHC class I molecule;
3) To use the identified EHV-1 peptide sequence to predict peptides from other horse pathogens that bind to this MHC class I molecule.

We have made good progress in the first nine months of this award.
Principal results have been in 3 areas:

1) Continued development of P-815 and RMA-S cell lines transfected with individual equine MHC class I genes.
We have now produced a total of 17 P-815 transfectants expressing MHC class I molecules. These include all 7 of the well-characterized MHC class I genes of the ELA-A3 haplotype, and putative peptide presenting MHC class I molecules of the ELA-A1, -A2, -A5, -A9, and -A10 haplotypes. These cell lines are now available for testing in cytotoxic lymphocyte (CTL) assays.

We have also produced 7 MHC class I gene transfectants in the RMA-S cell line that we are using in MHC stabilization assays using defined peptides (see section 2 below). The transfected equine MHC class I genes in RMA-S are 7-6 (ELA-A1 haplotype), 16*00101 (ELA-A2), N*00101 (ELA-A2), 5*00101 (ELA-A3 and other haplotypes, a non-classical MHC class I gene), 1*00101 (ELA-A3), 4*00101 (ELA-A3), and 1*00201 (ELA-A9). The horse MHC class I genes in these transfectants behave exactly like the endogenous mouse class I genes: the molecules are not expressed at 37 degrees but they do express when held overnight at room temperature.

2) Application of the RMA-S cell line peptide-MHC stabilization assay.
We have had success in stabilizing horse MHC class I genes in RMA-S cells using two peptides and genes from two MHC haplotypes. First, as proof of principle we used the Equine Infectious Anemia Virus (EIAV) peptide previously identified at Washington State University to stabilize expression of the 7-6 gene (RVEDVTNTAEYW). We were able to stabilize equine MHC class I expression in the RMA-S transfectants by adding this peptide to the cultures (Fig. 1). We tested 10 overlapping peptides surrounding the original 12-mer and defined critical amino acids at the left and right end that were essential for peptide binding and stabilization (data not shown). Irrelevant control peptides did not stabilize the 7-6 gene, and the 7-6 peptide did not stabilize the endogenous mouse MHC class I gene or other horse MHC class I genes in other RMA-S transfectants (data not shown).
Figure 1. Peptide stabilization of equine MHC class I genes transfected in mouse RMA-S cells. Cells: RMA-S cells transfected with the 7-6 MHC class I gene. Stabilization by the EIAV peptide RVEDVTNTAEYW. Green profile: immunofluorescence using directly labeled mouse anti-horse MHC class I monoclonal antibody CZ3. Red profile: negative control.

The EIAV peptide study was performed at Cornell. In parallel and using the same assay, research in Dr. Klaus Osterrieder’s lab in Berlin identified a peptide from human adenovirus that stabilized expression of the 16*00101 MHC class I gene of the ELA-A2 haplotype. This was done using a small peptide library and is the first identification of an unknown equine MHC binding peptide using the RMA-S stabilization assay.

3) Establishment of collaboration with the MHC peptide group at the La Jolla Institute of Allergy and Immunology.

The research group at the La Jolla Institute of Allergy and Immunology (LIAI) led by Dr. Alex Sette are world leaders in identifying MHC binding peptides using various binding assays and bioinformatics. They also work closely with Dr. Don Hunt’s group at the University of Virginia to identify peptide binding motifs using tandem mass spectrometry on peptides eluted from individual MHC molecules isolated from cells using antibody affinity columns. In January of 2013 Drs. Antczak and Tallmadge and Don Miller met with Dr. Sette and his colleagues at LIAI to discuss possible collaborations. We have begun to work together, exchanging data, protocols, and reagents, including new peptides for testing.

We have developed a unique collection of mouse cell lines transfected with individual horse MHC class I genes. We have also demonstrated how the RMA-S MHC-peptide stabilization assay can be used to identify peptides that bind horse MHC class I molecules. This approach will facilitate identification of immunogenic peptides that bind to equine MHC class I molecules for presentation to the T-cell receptor. This is a critical step in the design of new vaccines that stimulate cell-mediated immunity.

Our overall goals remain the same for Year 2. However, our emphasis has changed because of experimental successes and events of the past 9 months.

First, we plan to focus on using the transfected RMA-S cell lines to identify peptides that stabilize equine MHC class I molecules. This will require peptide libraries. We have received some peptides from the LIAI group that we have begun to test and we have ordered a peptide library that will be used with the RMA-S cell lines that we have established. This strategy has proved feasible in Year 1.

Second, we are planning a major experiment with the LIAI group to immunoprecipitate the ELA-A3.1 molecule from our P-815 transfected cell line, using our own anti-horse MHC class I monoclonal antibody, CZ3. Peptides would be eluted from the isolated class I molecule and sent to Don Hunt in Virginia for tandem mass spec analysis. This should identify the anchor motifs of the ELA-A3.1 molecule and allow us to use available bioinformatic search tools to find candidate peptides from equine herpesvirus and other horse pathogens that can bind to this MHC class I molecule. We would test binding using the RMA-S stabilization assay. We have begun generating the materials (anti-MHC class I antibody and P815 cells) to send to LIAI for affinity purification and peptide elution.

Finally, we will produce variants of the adenovirus peptide that stabilizes the 16*00101 MHC class I gene of the ELA-A2 haplotype, with the goal of identifying the anchor motifs for that molecule. Targeted CTL assays would be performed using the most promising peptides.
## 2013 Annual Report

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<tr>
<th>P.I.</th>
<th>Dr. Thomas Divers</th>
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<tr>
<td>Title</td>
<td>Etiology and Prevention of Equine Serum Hepatitis (Theiler’s Disease)</td>
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<tr>
<td>Project Period</td>
<td>1/1/13-12/31/14</td>
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<td>Reporting Period</td>
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PROJECT TITLE: Etiology and Prevention of Equine Serum Hepatitis (Theiler’s Disease)

PRINCIPAL INVESTIGATOR(S): Thomas Divers with Bud Tennant

Specific Aims: The three specific aims have not changed.

Specific Aim 1. Express clones of EHAV antigen sequences, purify the recombinant viral proteins and develop serologic tests for detection of EHAV antibodies. Expression of viral proteins and the development of immunosorbent antibody tests will be under the direction of Dr. Y.C. Change. The tests for EHAV antibodies are essential for the conduct of the second and third specific aims.

Specific Aim 2. The second specific aim will be to conduct a cross sectional study to assess the extent of exposure to EHAV. The target population will be North American horses. Consortium members will solicit participation of farms across the United States and Canada. Adult horses on the farm will be surveyed serologically to determine the extent of exposure to EHAV and the number of horses that are infected (EHA virus positive) or have been infected (EHAV antibody positive, EHA virus negative). The prevalence of EHAV infection in adult horses will be determined based on the presence of EHA viremia detected by RT-PCR, a procedure that currently is available, and the presence of EHAV antibodies to be detected by immunosorbent assays to be developed (Specific Aim 1).

Specific Aim 3. The third specific aim will be a prospective case control study, the purpose of which will be to determine the frequency of productive EHAV infection in successive new cases of acute hepatitis/Theiler’s disease. The risk of EHAV in Theiler’s disease cases will be compared to age and gender matched controls.

Specific Aim 1 - Studies and Results: Four genes of the Theiler’s disease associated virus (TDAV) were selected, expressed in E. coli, and the expressed protein purified. Western blot testing of the 4 proteins was performed using serum positive for TDAV by PCR and serum from a herd of horses that were tested for TDAV and were PCR negative. The expresses NS5A gene was found to provide the most accurate testing. This 30 Kd protein (30 Kd) was tested further by Western blot to establish the most appropriate concentrations of protein for Western blot testing. The NS5A protein will be used as the protein antigen in an ELISA for TDAV antibody detection. The projected completion of this aim is January-February 2014. Development of the ELISA is being performed in the laboratory of DR. Yung-Fu Chang.

Specific Aim 2 - Studies and Results: While the ELISA is being completed, collaborative arrangements are being established by Dr. Bruce Akey, Executive Director of the Cornell University Animal Health Diagnostic Center, to obtain serum samples from collaborating regional diagnostic centers from across North America to determine the prevalence of TDAV infection. Seven North American regional diagnostic laboratories will be recruited from the northeastern, southeastern, mid-western, southwestern and western regions of the United States and from an eastern and a western province of Canada. Serum samples from approximately 100 horses will be provided by each collaborating laboratory by using the residual serum from samples submitted for routine Equine Infectious Anemia testing. The geographic origin of each horse will be provided and age, gender, and breed will be obtained if possible.
Specific Aim 3: We are not aware of additional outbreaks of Theiler’s disease within the past six months. We have received samples from two isolated suspect cases both of which were PCR negative. A letter has been sent to 25 colleges and large private practices throughout the United States and Canada trying to recruit cases and age matched controls. We have continued to monitor the Nevada horses involved in the original outbreak and just recently (September 2013), identified two horses that remain PCR positive for TDAV, two years following antitoxin treatment and the hepatitis outbreak. Drs. Randy Renshaw and Ed Dubovi have performed the PCR testing on these recent samples.

Our 2013 findings further confirms that a low percentage of infected horses can become chronic carriers of the virus and these horses would be a risk for transmitting infection and possibly disease if used for equine serum/plasma production. If we find additional cases of acute hepatitis associated with TDAV infection we believe official testing will be needed for all equine plasma/serum products. Our intention is that any official testing will be performed at the Animal Health Diagnostic Center at Cornell University. There is so far no evidence of horizontal spread in the Nevada herd.

There are no modifications of the original plan. Drs. Tennant, Dr. Van de Walle, and Divers met with virologists at Rockefeller University and Columbia University and have submitted a joint NIH grant application with both groups to support further experimental studies of TDAV. The proposal includes the construction of a full length clone of TDAV, the establishment of its infectivity in vitro in cultured equine hepatocytes, and ultimately the cloned TDAV will be transfected (by direct hypodermic needle injection) into the liver liver of experimental horses. Koch’s postulates for TDAV would be fulfilled if a productive TDAV infection developed following transfection of the cloned TDAV.
Harry M. Zweig Memorial Fund
for Equine Research

2013 Annual Report

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<tr>
<th>P.I.</th>
<th>Dr. Norm Ducharme</th>
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<tr>
<td>Title:</td>
<td>An Exploratory Study into the Practical Application of a Regenerative Medicine Approach to Reconstruction of the Equine Upper Airway</td>
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<td>Project Period: Reporting Period:</td>
<td>1/1/13-6/30/14 1/1/13-12/31/13</td>
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Dr. Ducharme was granted an no cost extension through June 30, 2014. A progress report is provided (a final report will be included next year).
Project Title: An exploratory study into the practical application of a regenerative medicine approach to reconstruction of the equine upper airway

Principal Investigator: Norm Ducharme

Aims: To determine effective methods for the replacement of laryngeal cartilage using decellularized equine cartilage as treatment of cartilage abnormalities leading to airway obstruction. These regenerative medicine strategies require at first the development of efficient methods for the decellularization of equine cartilages, and subsequently the verification of performance of the scaffold based implants in a clinical model of laryngeal reconstruction.

The two specific aims of the study, to be conducted in two distinct and consecutive phases, were to:

1) Develop decellularization methods for the production of mechanically robust, geometrically accurate scaffolds from the equine cartilages while retaining the tissue specific ultrastructure and functional composition of the source tissues

2) Determine the ability of these acellular scaffold materials with and without bone marrow derived cells, to integrate and function as inductive templates for the reconstruction of the equine upper airway following partial resection of the epiglottis and arytenoid in vivo.

The first 11 months of the project focused on Aim 1, identifying the most effective protocol for decellularization of equine laryngeal cartilages.

Phase 1
Complications encountered and solved

1. Several issues affected the time to effectively start the Phase 1 and its duration. The delay was associated with the difficulty in collecting samples, but has been resolved.
2013 Progress Report

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<th>P.I.:</th>
<th>Dr. Lisa Fortier</th>
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<tr>
<td>Title:</td>
<td>Cellular Biomarkers of Early Cartilage Injury Measured with Multiphoton Imaging</td>
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Dr. Fortier was granted a no cost extension through June 30, 2014. A progress report is provided.
This proposal represents year 2 of a 2-year proposal submitted and approved during the 2013 Zweig funding cycle. However, it was determined that “There were limited funds available to support all meritorious proposals, which precluded a two year award for your study”. We have made significant progress in Aim 1 which has resulted in a manuscript in press in Osteoarthritis and Cartilage (Appendix 1). This proposal seeks to complete Aim 2 of the original proposal and adapt our ex vivo knowledge to an in vivo model.

Ex vivo validation of cartilage injury and MPM imaging - Our impact/MPM studies used freshly retrieved osteochondral blocks from the distal equine metacarpus because it is a highly prone area to injury in race horses. Results from these studies indicated that MPM could be used as an optical tool to detect subtle but measureable damage in live articular cartilage (Figure 3; Appendix 1). Sodium fluorescein, which is FDA approved for clinical use, was validated as an indicator of cell death. Despite the widespread clinical use of fluorescein for applications such as corneal ulcers, it had never been fully validated as a dead cell marker. This was an important step toward in vivo use where clinically approved materials are required. Cell death patterns were mapped in 3D and found to be circular or ellipsoid in shape. Cell death was increased in the superficial layers (Figure 4; Appendix 1) and in areas of matrix cracking. Matrix cracks were less than 1μm in diameter and were not detectable with other imaging tools, even research grade (7 Tesla) MRI.

The results from these studies provided ex vivo proof of concept that MPM can be used as an optical tool to measure cartilage damage in live tissue. We now seek to apply these methods to the in vivo situation.
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<tr>
<th>P.I.:</th>
<th>Dr. Lisa Fortier</th>
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<tr>
<td>Title:</td>
<td>Identification of the Optimal biologic to Enhance Endogenous stem cell recruitment and Homing for Facilitated Musculoskeletal Tissue Regeneration.</td>
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<td>Project Period:</td>
<td>1/1/12-6/30/14</td>
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Dr. Fortier was granted a no cost extension through June 30, 2014. A progress report is provided.
PROJECT TITLE: Identification of the Optimal biologic to Enhance Endogenous stem cell

Results: A significantly greater number of MSCs migrated toward the biologics than the neutral control (Figure 2; p = 0.03). On average, biologics attracted 78% of migrating cells. There were no significant differences in number of migrating cells between the biologics. The cell flux was significantly different between all the biologics and the neutral controls (Figure 3). However, within the biologics, BMAC resulted in significantly greater cell flux than PRP-1 (p = 0.028), as did PDGF (p = 0.028) but there were no other differences between biologics.

Discussion: The data indicates that all tested biologics have the ability to recruit MSCs. However, they did not significantly increase cell flux. Certain growth factors, such as IGF-1 and PDGF-BB, have been shown to have both chemotactic and/or chemokinetic effects on malignant mesothelioma cells (1). Although not measured in this experiment, all biologics would contain comparable levels of IGF-1, because of the basal level of IGF-1 in serum of both blood and bone marrow (2). In the groups tested, PRP-2 contained the highest concentration of platelets and therefore PDGF-BB, so it was hypothesized that PRP would result in the greatest chemotaxis of MSCs. There are many potential chemotactic and chemokinetic molecules in biologics, so it is overly simplified to attribute our results to only IGF-1 or PDGF-BB when all biologics resulted in increased recruitment of MSCs.

Significance:
Biologics such as PRP or BMAC can be used as chemotactic agents to recruit MSCs to a site of injury. This information will reduce the need and associated risks and costs associated with direct stem cell delivery.

References

Acknowledgements
Harry M. Zweig Fund (LAF) Supported by the Empire State Stem Cell Fund through New York State Department of Health Contract # C028097. (HLH)
2013 Progress Report

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<th>P.I.:</th>
<th>Dr. Tracy Stokol</th>
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<tr>
<td>Title:</td>
<td>The Role of Platelets in the Pathogenesis of Equid Herpes Virus Type-1 Infection</td>
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Dr. Stokol was awarded an additional no cost extension through December 31, 2014. An interim report is provided (a final report will be included next year).
PROJECT TITLE: The Role of Platelets in the Pathogenesis of Equid herpes virus type-1 Infection

PRINCIPAL INVESTIGATOR(S): Dr. Tracy Stokol

The aims have not been modified. We have requested and received an additional no-cost extension to continue with the studies.

Studies & Results

We have requested an additional (and final) no-cost six month extension for the above Harry M. Zweig Memorial Fund for Equine Research Program grant through December 31, 2014 in the hopes of finishing this study.

The good progress continues with this grant. We have two manuscripts in preparation, which are outlined below:

1. Thus far, we have found that EHV--1 does activate platelets, upregulating P selectin and causing microvesiculation. We have only recently obtained an EHV--1 mutant from Dr. Osterrieder that we need to test a hypothesis on how EHV--1 is activating the platelets. This mutant virus is being amplified and we hope to complete the experiments over summer. An abstract on this study has been accepted for presentation at the 7th Symposium on Hemostasis in North Carolina in May 2014 and was also submitted for presentation for the annual meeting of the International Society of Thrombosis and Haemostasis (meeting in Wisconsin in June, 2014).

2. Using a microfluidic device, we have now also shown that EHV--1-infected endothelial cells upregulate P selectin and recruit unactivated platelets in a P selectin--dependent manner. We have more conditions to test before submission of this manuscript and this will likely take several more months (likely into Fall).

3. Through these Zweig--funded research efforts, we have secured external funding from the Grayson Jockey Club to test whether available antiplatelet drugs can prevent EHV--1 from activating platelets. This new study is set to begin in May 2014. With this additional 6 month no--cost extension until 31st December 2014, we will have sufficient time to complete all the proposed experiments and submit the manuscripts.
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<th>P.I.:</th>
<th>Dr. Bettina Wagner</th>
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<tr>
<td>Title:</td>
<td>Innate Immune Mechanisms and T-Cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses</td>
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<td>Reporting Period:</td>
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PROJECT TITLE: Innate Immune Mechanisms and T-Cell Responses to Equine herpes virus Type 1 in Latently Infected and Naïve Horses

PRINCIPAL INVESTIGATOR(S): Dr. Bettina Wagner & Dr. Gillian Perkins

**Aim 1**, we will experimentally infect horses with Ab4 wild type and Ab4ΔORF1/2 mutant strains. We will evaluate viral shedding, clinical signs of disease, the ability to induce robust cellular adaptive immunity and protection in experimentally infected horses. This experimental infection study was proposed for November 2013.

**Current status:** The EHV-1 free horses for Aim 1 have been imported from Iceland in April 2013 and are currently kept at the Equine Drug Testing Facilities. They have been monitored for their EHV-1 free status before and after they arrived at Cornell. EHV-1 specific antibody testing and cellular immune assays are performed at least on a monthly basis and the horses are evaluated clinically at least once a week. The infection experiment is planned for November 2013. An amendment to Dr. Wagner’s animal protocol has been submitted to IACUC and an update of the MUA to include *in vivo* use of the Ab4 wild type and Ab4ΔORF1/2 mutant viruses has been sent to the Institutional Biosafety Committee. There were some concerns about the horses being infected with the mutant virus. Dr. Wagner and Dr. Perkins met with Frank Cantone, Deb Dwyer and Dr. Parrish last week to come to an agreement on the procedures. We are working together on an SOP and expect that this will be solved very soon to grant approval from both committees for the infection experiment.

**Plans for the next year:** We will proceed with the experimental infection study in November 2013 as proposed. We will follow the immune responses of these horses and then re-infect them in 2014 to test for protection from re-infection. We expect to determine whether the EHV-1 Ab4ΔORF1/2 mutant is a candidate for an effective EHV-1 vaccine that induces high levels of cellular and humoral immunity, reduces clinical signs and can offer increased protection compared to currently available EHV vaccines for horses. We also expect to gain new insights about the longevity of antibody titers and cellular immunity after infection with the neurogenic EHV-1 Ab4 and its mutant strain.

**Aim 2,** we will investigate underlying mechanisms that result in immune modulation by ORF1/2, UL49.5 and ICP0 genes. Cells will be obtained from clinically healthy horses for *in vitro* analysis. We will use PBMC from non-infected/EHV-1 naïve (n=8), vaccinated (n=8), and horses with a known history of previous natural infection (n=8). We will analyze: (1) Differences in cytokines induction between the groups and between the Ab4 wild type and mutant strains will be analyzed by multiplex analysis; (2) Further analysis of IFN-γ and IL-4 producing T-cells and non-T-lymphocytes induced by Ab4 wt and the OFR1/2 mutant will be performed by flow cytometry; (3) We will identify EHV-1 infected cells *in vitro* and *ex vivo*; and (4) Enrichment of EHV-1 infected cells will be performed to identify latent EHV-1 infection in clinically healthy horses.

**Results:** Part 1 of Aim 2 is completed for naïve and vaccinated horses. The major findings for these two groups are: (i) ORF1/2 gene deletion mutant reduced the induction of type I interferon as well as pro- and anti-inflammatory cytokines compared to Ab4 in both groups (Fig. 1); (ii) the UL49.5 mutant decreased type I interferon induction in both groups; (iii) the ICP0 mutant had no effect on cytokine profiles in PBMC from vaccinated horses, except for a relatively small up-regulation of CCL2.
We consider anti-viral IFN-α responses as a ‘danger signal’ for other not yet infected cells. IFN-α is produced after binding of viral DNA to endosomal Toll-like receptor 9 within the infected cells. Type I interferons have been shown to have protective effects on non-infected cells. However, less ‘danger signal’ induced by the ORF1/2 and UL49.5 mutants also suggest that these viruses either infect cells less efficiently than the parent Ab4 strain or replicate less after infection.

![Graph](image)

represents one horse. We were able to include more horses in the experiment. All statistical comparisons are to the parental (wild-type) Ab4 virus.

A manuscript for part 1 of Aim 2 (multiplex analysis was submitted to Veterinary Research and is currently under revision (Goodman LB, Wimer C, Damiani A, Freer H, Osterrieder N, Wagner B. Immune regulation by equine herpesvirus type 1 (EHV-1) ICP0, UL49.5, and UL56 proteins in equine peripheral blood mononuclear cells. Vet Res., under revision). The reviewers asked to include the information that we target in part 2 of Aim 2 of this project. We are currently performing the missing experiments and will resubmit the manuscript after the data analysis is completely finished.

Part 2 of Aim 2 is currently ongoing. Our preliminary data showed that the Ab4ΔORF1/2 mutant increased IFN-γ in T-cells and non-T-cells compared to wild type Ab4. We also observed an increase in IL-4 and IL-10 production by CD4+T-cells and non-T-cells in cells that were infected with the mutant, while Ab4 did not induce any detectable IL-4 or IL-10. This suggested that the deletion of the ORF1/2 genes reversed the immune-suppressing effect that Ab4 generally has on the immune response.

During the first months of this project, we tested additional horses and used other cell markers to better characterize the IFN-γ, IL-4 and IL-10 producing lymphocytes after *in vitro* infection with Ab4 and the different mutants. The most striking result was that the cytokine-producing cells after infection with the ORF1/2 mutant are composed of a variety of cell populations. As expected, a number of CD4 and CD8 cells produced IFN-γ (similar to Ab4). In addition, the ORF1/2 mutant induced IFN-γ and IL-4 production in cells that were negative for T-cell markers and cell surface positive for IgM and IgG1 which are the major B-cell markers in horses. This was an unexpected finding (and challenging a dogma). We are currently investigating these cells in more detail to better identify them and to confirm that they are indeed B-cells and not just cells that bound IgM or IgG1 via cell surface Fc-receptors. The results will be included into the manuscript that is mentioned above.

**Plans for the next year:** We plan to finish the experiments for part 1 and 2 of Aim 2 in the next few months and after cells from infected horses are available. Parts 3 and 4 of Aim 2 will be performed next year as planned.
Significance of the findings and their potential impact

The goal of this project is to provide proof-of-principle for the efficacy of a new vaccine candidate (Ab4ΔORF1/2) for EHV-1. Based on our accumulated *in vitro* data, the Ab4ΔORF1/2 deletion mutant is a very promising EHV-1 vaccine candidate with a clear potential to lead to protection against neurological disease (myeloencephalopathy) and, in the long-term, an overall better protection against EHV-1 in horses.
**P.I.:** Dr. Bettina Wagner  
**Title:** A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates  
**Project Period:** 1/1/12-12/31/13  
**Reporting Period:** 1/1/13-12/31/13
PROJECT TITLE: A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates

PRINCIPAL INVESTIGATOR(S): Bettina Wagner & Gillian Perkins

In the project preceding this proposal, we investigate the hypothesis that antigen-specific activation of basophils and production of the cytokine interleukin-4 by activated basophils is a major pathway to induce antibody production in foals after birth. The hypothesis was tested by two specific aims.

In Aim 1, we proposed to generate antibody/EHV-1 antigen complexes that mediate activation of basophils in vitro. Five EHV-1 antigens previously known to induce antibody responses were expressed and tested. Basophils were isolated, loaded with antibody and activated with EHV-1 antigens. Basophil activation was evaluated by cytokine induction. In this aim, we also proposed to develop a sensitive EHV-1 antigen-specific multiplex assay with the goal to improve the evaluation of EHV-1 antibody responses in horses.

Results: We have expressed four of the five EHV-1 antigens (gB, gC, gD, gG). Expression of the fifth antigen (gp2) which is a very large protein was found to be challenging but is still ongoing. The first two expressed antigens (gC and gD) have been administered to the neonatal foals in June 2012 (see Aim 2). They were also used to perform in vitro experiments to further test the underlying principle of this project. Basophils were enriched from peripheral blood, IgE was loaded on the cells by overnight incubation and stimulation with antigen was performed to induce IL-4 secretion from basophils. This confirmed that high concentrations of B-cell stimulatory IL-4 can be induced from basophils in an antigen-specific way and by using our ‘immune tools’. We determined the optimal IgE and EHV-1 antigen concentration and continued with the in vivo experiment (Aim 2).

The gC and gD antigens were also used to develop a multiplex assay for detection of EHV-1 antibodies in serum. The multiplex assay uses recombinant gC and gD antigens to simultaneously measure antibodies to both surface glycoproteins. Each of the EHV-1 antigens is coupled to a specific fluorescent color-coded bead to distinguish between the individual assays. We used 58 pretested equine sera with SN-values between <2 to 768 to validate the multiplex assay. SN-titers and multiplex results highly correlated (rSpearman = 0.87). The multiplex assay allows for a more accurate, faster (hours instead of days) and fully quantitative determination of antibodies to EHV-1 than the SN-test. The EHV-1 multiplex assay will be offered to veterinarians through the Animal Health Diagnostic Center in the near future. For this project, the assay was used to evaluate all serum and nasal secretion samples obtained from foals enrolled in Aim 2 and the assay was modified to perform an analysis of equine immunoglobulin isotypes against EHV-1.

Aim 2 was to test the potential of a novel neonatal vaccination strategy in foals directly after birth. We gave IgE to foals from EHV-1 naïve mares within the first 6 hours after birth. At day 2, foals obtained EHV-1 antigen to induce antibody production. After weaning, foals were experimentally infected with the EHV-1 strain NY03. Clinical signs, viral loads and EHV-1 specific immunity was evaluated.
Results: We performed the first part of this Aim in foals that were born in June 2012 at Cornell. Their dams were imported to Cornell in February 2012 and came from Iceland. Iceland is free of EHV-1. The EHV-1-free status of the horses was maintained by quarantine and isolated housing at Cornell. Consequently, the foals did not obtain EHV-1 specific antibodies from their dams via colostrum. This allowed us to determine the foal’s endogenous EHV-1 immune response in the absence of maternal EHV-1 specific immunity. Fifteen foals were placed in 3 groups. Group 1 received IgE orally during the first 3 hours after birth and EHV-1 antigen on day 2 of life. Group 2 received EHV-1 antigen on day 2 but no IgE. Group 3 was not treated at birth. Blood and nasal swab samples were constantly taken from the foals to monitor their direct response to the treatment at birth. IL-4 expression by neonatal foal basophils was confirmed after birth. A transient antibody response was initiated during the first week of life in foal group 1 that received IgE and antigen. This response was significantly higher than in the other two foal groups.

After weaning at seven months of age, all 15 foals were experimentally infected with EHV-1 (NY03 strain). The time point was chosen because weaning is a stress factor for the foals and EHV-1 transmission has often occurred at this time. The experimental infection study with EHV-1 showed a significant decrease of the peak fever response in group 1 (IgE/Ag) compared to both other groups and a lower but not-significantly lower clinical score. Groups 1 (IgE/Ag) and 2 (Ag only) also produced significantly higher amounts of EHV-1 specific antibodies compared to group 3 (not treated at birth). Cellular immunity did not differ between the three groups. The finding was expected because the treatment at birth targets B-cell activation and antibody production in a T-cell independent way.

How can these results be interpreted? We could show that our non-classical ‘EHV-1 vaccination’ approach of neonatal foals at birth initiated a short-term immune response at birth. However, increased antibody production and decreased clinical signs after experimental EHV-1 infection suggested that the initiation of EHV-1 immunity at birth, despite of transient antibody induction, provided significant protection to the foals seven months later. In this project, we now hypothesize that our neonatal vaccination induced B-cell memory at birth that initiates a more rapid and robust antibody response and less severe clinical signs to EHV-1 infection several months later.

The data above have not yet been published. Currently, foals are first vaccinated with classical vaccines at 4-6 months of age. Classical vaccines do not work effectively when used earlier in life. Neonatal vaccines do not exist for foals. The discovery of a vaccination mechanism which initiates an immune response in neonates and induces protective immunity (although not fully protective in our first approach) provides a promising novel strategy to prevent transmission of pathogens to very young foals. The approach has thus been submitted first for invention disclosure with CCTEC. Presentation and publication will follow after disclosure of the invention.
APPENDIX B

SUMMARY OF 2013 EXPENDITURES

2013 Research Awards  $471,394
2014 Public Relations and Administrative Budget  $25,500
2013 Incentive Awards  $7,352

Total Expenditures:  $504,246
APPENDIX C

POSTER SESSION PRESENTATIONS
November 13, 2013
Cornell University College of Veterinary Medicine
Equine researchers present to Zweig Fund committee

Faculty, graduate students, and researchers presented on the College's many equine-related research projects in a series of posters and lectures on November 13, 2013, at the Veterinary Education Center. Members of the committee administering the Harry M. Zweig Memorial Fund for Equine Research attended the event to see the success of currently funded projects and to learn about new research going on at the College.

Speakers included Tracy Stokol, Associate Professor, Department of Population Medicine & Diagnostic Sciences, Jonathan Cheetham, Principal Research Scientist - Zweig Principal Research Scientist (2012-2013), Department of Clinical Sciences, Gerlinde Van de Walle, Assistant Professor, Baker Institute for Animal Health, and Thomas Divers, Professor and Bud C. Tennant, Professor, Department of Clinical Sciences with their respective lectures on “Platelets and Equine Herpes Virus Type 1 Infection”, “Diagnosis of Poor Performance in Racehorses”, “The Role of Equine Mesenchymal Stem Cells during Equine Herpes Virus Type -1 Infection”, and “Equine Hepatitis Virus Discovery and its Potential Importance to Equine Health.” A reception followed outside the lecture hall, where faculty, students and staff mingled with the Zweig Committee to talk about the research presented at the lecture and posters on display.

Zweig Committee members also met with the student chapter of the American Association of Equine Practitioners (SC-AAEP) at Cornell University and their faculty advisor, Gillian Perkins, DVM, Diplomate ACVIM, to partake in an informal discussion about their careers, work-life balance and where they see the profession of equine veterinary medicine going in the future. Questions about externships and internships, practice experience, etc. were among topics discussed, and both the committee and the students enjoyed the interaction.

The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry Zweig, a distinguished veterinarian, and his numerous contributions to the state’s equine industry. In 1979, by amendment to the pari-mutuel racing and wagering law, the New York State legislature created the Harry M. Zweig Memorial Fund for Equine Research to promote equine research at the Cornell University College of Veterinary Medicine. The Harry M. Zweig Committee was established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians. The Fund contributes a percentage of its revenue to support a variety of equine-related research. The Fund is proud to support the Harry M. Zweig Memorial Fund for Equine Research. This first-rate research helps to provide protection and preventative planning for the equine industry, which in turn helps to ensure a healthy and positive future for the horse racing industry.

The committee administering the fund always includes the chairman of the New York State Racing and Wagering Board or his designee, the dean of the College of Veterinary Medicine at Cornell or his designee, a member or the executive director of the Agriculture and New York State Horse Breeding Development Fund, a member or the executive director of the New York State Thoroughbred Breeding and Development Fund, and at least five New York State breeders, owners, trainers, or veterinarians in equine practice. Dean Michael Kotlikoff currently serves on the committee, representing the College and its many researchers who have received the Fund’s support for research projects advancing equine health and athleticism.
5th Annual Harry M. Zweig Memorial Fund for Equine Research Poster Session & Talks
Cornell College of Veterinary Medicine
Ithaca, New York

FEATURING SPEAKERS FROM CORNELL’S COLLEGE OF VETERINARY MEDICINE

Wednesday, November 13, 2013 • Veterinary Education Center Atrium – Lecture Hall II – 3:00pm-4:30pm
Everyone is welcome to attend one Presentation or all/Reception.

3:00PM Welcome!
Dr. Joel D. Baines Associate Dean Research & Graduate Education

3:05PM Tracy Stokol -- Associate Professor
“Platelets and Equine Herpes Virus Type-1 Infection”

3:25PM Jonathan Cheetham -- Principal Research Scientist - Zweig Principal Research Scientist (2012-2013)
“Diagnosis of Poor Performance in Racehorses”

3:45PM Gerlinde Van de Walle -- Assistant Professor
“The Role of Equine Mesenchymal Stem Cells during Equine Herpes Virus Type-1 Infection”

4:05PM Thomas Divers -- Professor & Bud Christopher Tennant -- Professor
“Equine Hepatitis Virus Discovery and its Potential Importance to Equine Health”

4:30-6:00pm Wine and Cheese Reception- Atrium – outside Lecture Hall II
All are Welcome!
College of Veterinary Medicine and the
Harry M. Zweig Memorial Fund for Equine Research

Poster Session & Talks

Wednesday, November 13, 2013
3:00pm-6:00pm

Atrium Veterinary Education Center
Welcome to the 5th Annual Zweig Poster Session to celebrate over 30 years of collaboration and support between Cornell College of Veterinary Medicine, and the Harry M. Zweig Memorial Fund for Equine Research.

The posters have been created by faculty, graduate students and residents who have been a recipient of an award from the Fund, or are working on equine related research.

We would like you to enjoy looking at these posters and please feel free to ask questions. We would also like to thank the presenters for taking the time to join us in celebration of the the Harry M. Zweig Memorial Fund for Equine Research.

Thank you for your participation.

Dr. Joel D. Baines, Associate Dean - Research and Graduate Education

Laura A. Mathews, Secretary for the Zweig Committee
Global nutritional utilization profile determined using phenotype microarrays reveals differences between nutrient requirements of pathogenic and non-pathogenic Leptospira sp.

Joy Scaria, YongGuo Cao, Jenn-Wei Chen, Yung-Fu Chang

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

Presenting Author: Joy Scaria

The genus Leptospira is composed of pathogenic and non-pathogenic species that differ in habitat and host range. Members of Leptospira interrogans are pathogenic while L. biflexa is a saprophytic species. The nutritional requirements of Leptospira is key factor that determines the virulence of the strains. To understand the key differences in nutritional requirements of pathogenic and non-pathogenic Leptospira sp, using Biolog Phenotype microarrays (PM), we compared the global nutritional requirements of L. interrogans and L. biflexa. Biolog phenotype microarray is high-throughput assay that screens for over 700 nutrients. Therefore, the complete phenotype profile of the strains was obtained by screening against PM panels 1 through 8. For PM 1through 8, 25% increase in signal intensity over negative control was considered positive. Of the 730 nutrients tested, 300 nutrients were utilized by at least one strain. The most preferred carbon and nitrogen source for L. interrogans and L. biflexa were tween 20, tween 60 and tween 80. These comparisons also revealed key differences in nutritional requirements of both species. While L. interrogans utilized L- serine, L-alanine, D-fucose, L-sorbose, L-Rhamnose and turanose, L. biflexa could not utilize these nutrients. Likewise, while L. interrogans could utilize di-peptide nutrients such as His-Asp, His-Gly, His-Lys, and His-Met, L. biflexa did not utilize these peptides as nutrient sources. These differences in nutrient utilization we detect in this study might have implications in the in vivo survival of L. interrogans.
(#2) Development of an ELISA using recombinant LigACon4-7.5 as antigen for the diagnosis of equine leptospirosis

Weiwei Yan¹, Muhammad Hassan Saleem¹, Patrick McDonough¹, Sean P. McDonough², Thomas J. Divers³, Yung-Fu Chang¹*

¹Population Medicine and Diagnostic Sciences, ²Department of Biomedical Sciences, and ³Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

Presenting Author: Weiwei Yan

Leptospira immunoglobulin (Ig)-like protein (Lig protein) is a novel family of surface-associated proteins in which the N-terminal 630 amino acids are conserved. In this study, we truncated the LigA conserved region into 7 fragments comprising the 1st to 3rd (LigACon1-3), 4th to 7.5th (LigACon4-7.5), 4th (LigACon4), 4.5th to 5.5th (LigACon4.5-5.5), 5.5th to 6.5th (LigACon5.5-6.5), 4th to 5th (LigACon4-5) and 6th to 7.5th (LigACon6-7.5). All 7 recombinant Lig proteins were screened using a slot shaped dot blot assay for the diagnosis of equine leptospirosis. Our results showed that LigACon4-7.5 is the best candidate diagnostic antigen in a slot shaped dot blot assay. LigACon4-7.5 was further evaluated as an indirect ELISA antigen for detection of Leptospira antibodies in equine sera. This assay was evaluated with equine sera (n=60) that were microscopic agglutination test (MAT)-negative and sera (n= 220) that were MAT-positive to the 5 serovars that most commonly cause equine leptospirosis. The indirect ELISA results showed that at a single serum-dilution of 1:250, the sensitivity and specificity of ELISA were 80.0% and 87.2% respectively compared to MAT. In conclusion, an indirect ELISA was developed utilizing a recombinant LigA fragment comprising the 4th to 7.5th repeat domain (LigACon4-7.5) as a diagnostic antigen for equine leptospirosis. This ELISA assay was found to be sensitive, specific and concurred with the results of the standard MAT.
Variable response of cartilage to impact between joints and implications for the development of post traumatic osteoarthritis

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Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

Presenting Author: Kira D. Novakofski

Introduction. Osteoarthritis (OA) affects multiple joints. Studies typically evaluate a single joint or subset of joints, and the results are extrapolated to other, unrelated joints. Yet, the incidence of OA is not the same between different joints. Susceptibility to OA may be due to differences between joints in chondrocyte distribution, basal gene expression, and resiliency to injury. The hypothesis for this study is that cartilage in different joints is inherently different and responds dissimilarly to injury.

Methods. In the present study, cartilage explants (diameter=6 mm) were aseptically harvested from young adult horses (ages 2.5-4 yrs, n=4). Explants were removed from eight major joints, including the shoulder (SH), elbow (EL), carpal (CA), metacarpophalangeal (MC), patellofemoral (FP), tarsal (TA), metatarsophalangeal (MT), and proximal interphalangeal (PP) joints, and were injured by compressing with 30 MPa within 1 second. Cell density, cell death, and gene expression were quantified.

Results. There were no statistical differences in overall cell density as a function of depth, between joints. However, the density of chondrocytes within the superficial zone was different between joints (p=0.002). Overall injured samples had on average significantly higher cell death (17.55%, SE=0.01) than non-injured samples (4.62%, SE=0.01, p<0.0001). Injury and joint had significant effect on cell death (p<0.0001). Basal collagen type 2α1 (COL2A1) expression in controls was 13.9x lower in FP (p=0.0114) and 9.6x lower in SH (p=0.0460) than PP. Basal cartilage-derived retinoic acid-sensitive protein (CD-RAP) expression in controls was 16.3x higher in PP (p=0.0098) and 14.6x higher in TA (p=0.0202) than FP. After injury, the change in CD-RAP expression increased 204% in FP over MC (p=0.0460).

Conclusion. Characteristics in normal cartilage and the response to injury in eight joints were compared to better understand the pathogenesis of PTOA. Between joints, differences were found in basal characteristics, including cell density and gene expression, and in response to injury, including cell death and gene expression. These results suggest that different joints may have varying susceptibility to post-traumatic OA pathogenesis.

GWAS establishes correlation between growth and laryngeal neuropathy in the Thoroughbred Horse.


Department of Biomedical Sciences, & Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

Presenting Author: Adam Boyko

Equine recurrent laryngeal neuropathy (RLN) is a bilateral mononeuropathy with an unknown pathogenesis that significantly affects performance in Thoroughbreds. A genetic contribution to the pathogenesis of RLN is suggested by the higher prevalence of the condition in offspring of RLN-affected than unaffected stallions. To better understand RLN pathogenesis and its genetic basis, we performed a genome-wide association (GWAS) of 282 RLN-affected and 268 control Thoroughbreds.

We found a significant association of RLN with the LCORL/NCAPG locus on ECA3 previously shown to affect body size in horses. Using height at the withers of 505 of these horses, we confirmed the strong association of this locus with body size, and demonstrated a significant phenotypic and genetic correlation between height and RLN grade in this cohort. Secondary genetic associations for RLN on ECA18 and X did not correlate with withers height in our cohort, but did contain candidate genes likely influencing muscle physiology and growth: myostatin (MSTN) and integral membrane protein 2A (ITM2A).

The linkage between body size and RLN suggests that selective breeding to reduce RLN prevalence would likely reduce adult size in this population. Our results do not preclude the possibility of modifier loci that attenuate RLN risk without reducing size or performance, or that the RLN risk allele is distinct but tightly linked to the body size locus on ECA3. This study is both the largest body size GWAS and the largest RLN GWAS within Thoroughbred horses to date, and suggests that improved understanding of the relationship between genetics, equine growth rate and RLN prevalence may significantly advance our understanding and management of this disease.
Identification of the Optimal Biologic to Enhance Endogenous Stem Cell Recruitment

Hannah L. Holmes\textsuperscript{1}, Brooke Wilson\textsuperscript{1}, Julian P. Goerger\textsuperscript{2}, Jesse L. Silverberg\textsuperscript{3}, Lisa A. Fortier\textsuperscript{1}

\textsuperscript{1}Department of Clinical Sciences, College of Veterinary medicine, Cornell University, Ithaca NY \textsuperscript{2}Department of Biomedical Engineering, College of Engineering, Cornell University, Ithaca NY \textsuperscript{3}Department of Physics, Cornell University, Ithaca NY, United States

Presenting Author: Hannah Holmes

Bone marrow derived mesenchymal stem cells (MSCs) demonstrate promise for musculoskeletal regenerative therapy. Unfortunately, this therapy is not widely available because obtaining autologous stem cells is costly, time consuming, and not presently approved by regulatory agencies. An alternate approach to the direct delivery of stem cells is to exploit the concept of in situ tissue engineering whereby methods are used to recruit the body’s endogenous reservoir of local stem cells. Biologics such as bone marrow aspirate concentrate and platelet rich plasma (PRP) have successfully enhanced recovery from musculoskeletal injuries. These biologics contain growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor- β (TGF-β) which could act as chemotactics for stem cells. The purpose of this study is to identify the optimal biologic for recruitment of stem cells. Our hypothesis is that PRP will result in the greatest migration of MSCs because of the milieu and concentration of growth factors contained in PRP. A microfluidics device was used to compare the ability of biologics to recruit stem cells. The device was set up so the cells could choose between migrating towards 10% FBS media or one of the biologics and then imaged over the course of twenty four hours. Time-lapse images were analyzed by tracking the migratory patterns of the individual cells. The data indicates that all tested biologics have the ability to recruit MSCs. Biologics such as PRP or BMAC can be used as chemotactic agents to recruit MSCs to a site of injury. This information will reduce the need and associated risks and costs associated with direct stem cell delivery.
Development of an Equine Model of Post-Traumatic Osteoarthritis

Michelle L. Delco, DVM\textsuperscript{1}, Lawrence J. Bonassar, PhD\textsuperscript{1}, John G. Kennedy, MD\textsuperscript{2}, Rocky Tuan, PhD\textsuperscript{3}, Peter G. Alexander, PhD\textsuperscript{3}, Lisa Fortier, DVM, PhD\textsuperscript{1}.


Presenting Author: Michelle Delco

Joint injury and resulting osteoarthritis (OA) are common causes of poor performance and shortened athletic lifespan in equine and human athletes alike\textsuperscript{1}. Evidence suggests that in both species, direct trauma to the cartilage surface plays a pivotal role in initiating and perpetuating the degenerative process known as post-traumatic osteoarthritis (PTOA.) However, the relationship between mechanical injury to cartilage and the biology of early PTOA remains poorly understood\textsuperscript{2}. One reason for this knowledge gap is the limitation of current models to accurately simulate early PTOA in live animals. The objective of this study was to validate the use of a high-speed, spring loaded impacting device to create an impact injury to equine talar cartilage. We demonstrate that this device is able to deliver an injury to cartilage that would be expected to initiate PTOA in vivo.

Tissue collection and impact injury

Right and left tali were harvested from normal horses (n = 6, ages 2-11 years) immediately following euthanasia, and incubated in phenol-free MEM media buffered with HEPES. Osteochondral (OC) blocks were mounted in an adjustable vice grip, which allowed the articular surface to be positioned perpendicular to the direction of impact. The joint surface of the talus was impacted in regions corresponding to the highest incidence of naturally occurring osteochondral lesions in humans\textsuperscript{3} (Figure 1) using a custom-made spring loaded impacting device\textsuperscript{4} (Figure 2a). Displacement of the impactor tip was measured using a linear variable differential transformer (LVDT). Impact force was measured by a load cell within the impacting device. Cartilage thickness measurements and impact area were measured to calculate stress-strain data. Three tip designs and 6 spring tensions were compared.

Multiphoton imaging and histology

Impacted OC blocks were incubated in media for approximately 1 hour, then full-thickness cartilage sections containing the impact or control site were cut off the bone and placed in 1 μM sodium fluorescein for at least 15 minutes prior to imaging to stain dead cells. Samples were imaged with multiphoton microscopy using a Ti:sapphire laser at 780 nm excitation. Images were acquired at the articular surface in the transverse plane. Dead cells and extracellular matrix (ECM) microcracks were quantified, and the difference
between control and impacted cartilage was assessed using a two sample T-test. Impacted and control cartilage samples were fixed in 4% paraformaldehyde, then sectioned and stained with H&E and safranin O/fast green to assess morphology and relative proteoglycan content.

**Mechanical data**
By adjusting spring tension and varying tip design (diameter and radius of curvature), maximum stresses ranging from greater than 160 MPa to less than 40 MPa were achieved (Figure 2b). Impact times averaged 1.7 ms (±0.04 ms). The spring-loaded impactor, set to a spring spacing of 15 mm, delivered a consistent maximum stress averaging 46 MPa (± 1.5). Strain was calculated to be 0.54 (± 0.1) with a strain rate of 435%/sec (±32).

**Cell death, microcracking and histology**
Impacted samples contained more dead cells than control samples (Figure 3). The difference in cell death between control and impacted samples reached significance between 30 and 60 microns from the articular surface. Although cell death appeared more widespread when impacting with the flatter tip of larger diameter, cartilage microcracking was less common than with the small-diameter tip. Histologic examination revealed articular surface fibrillation and matrix microcracking in impacted samples (Figures 3 d, f).

Evidence suggests that the *rate* of loading may be more important than the *magnitude* of applied force during traumatic injury of cartilage.² Range are required to exceed physiologic loading in vivo. Here, we demonstrate these thresholds for impact injury are achievable using the described spring-loaded device. The impactor can deliver a repeatable, measurable injury to the articular surface of the equine tali resulting in fissures in the ECM and cell death within one hour of impact injury.

Most live-animal studies of equine OA involve creating a chip fracture within a joint. While these systems are useful to study a particular disease syndrome (e.g. carpal chip fractures in racehorses) they are not specific to cartilage damage. Therefore, they do not allow investigation of the early underlying mechanisms by which mechanical trauma to cartilage initiates PTOA. To this end, we are developing a rapid impact model utilizing the cartilage of the equine talus. Similar to the equine talocrural joint (upper hock joint), the human talocrural joint (ankle) rarely suffers from spontaneous OA. The human ankle is the most frequently injured joint in athletes, with PTOA commonly developing secondary cartilage injury.¹ In the horse, the low incidence of spontaneous OA and large joint size (allowing straightforward intraarticular placement of the impactor during arthroscopic surgery) make the talocrural joint ideal for modeling PTOA in vivo.

The current ex vivo results set the stage for in vivo studies. Development of an equine talocrural cartilage impact model, which creates cartilage injury similar to that expected to cause PTOA, will allow us to investigate early events in PTOA at the cellular and microstructural level over time in live animals.
Use of this model will allow for preclinical testing of preventative strategies such as exercise modification, medications, regenerative therapies, etc. to minimize the long-term development of PTOA.

Acknowledgments: Funded by the Harry M. Zweig Fund for Equine Research (LAF) and NIH grant number T32RR007059 from the National Center for Research Resources (MLD). The authors gratefully acknowledge Alex VanSlyke for assistance with multiphoton imaging.

References:

Figures:
The Cornell Icelandic horses were recently imported and are immunologically naive for many horse pathogens that occur in the US. Our main research focus is on immunity and vaccine development to equine herpesvirus type 1 (EHV-1). Two vaccine studies funded by the Harry M. Zweig Fund for Equine Research at Cornell are currently ongoing. These studies aim to develop an EHV-1 vaccine for neonatal foals and also to evaluate vaccine candidates that provide better protection against neurological disease induced by EHV-1. We are also investigating the immune mechanisms that lead to clinical allergy by using Culicoides hypersensitivity, an allergy mediated by Culicoides-specific IgE, as a natural disease model. At Cornell, the horses will be naturally exposed to Culicoides, with 50-70% of the imported horses developing the disease after being exposed and sensitized. Samples from the horses are further used for various small clinical and diagnostic projects including testing of Lyme vaccines, colostrum studies and the development of new diagnostic assays for EHV-1 and WNV.
A novel strategy to boost antibody production to equine herpesvirus type 1 (EHV-1) in neonates

Gillian Perkins, Laura B. Goodman, Susanna Babasyan, Heather Freer, Alison Keggan, Amy Glaser, Sigurbjorg Torsteinsdóttir, Vilhjálmur Svansson, Sigríður Björnsdóttir, and Bettina Wagner

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Presenting Author: Gillian Perkins

Despite widely used vaccination, EHV-1 outbreaks continue to occur in the US. This emphasizes the need to develop alternative vaccination strategies to better protect horses against EHV-1 and equine industries from economic losses induced by EHV-1 outbreaks. Most horses are first infected with EHV-1 either as foals or weanlings and remain latently infected for life. Successful early-in-life vaccination can substantially decrease viral transmission and clinical EHV-1 outbreaks in horses. Unfortunately, neonatal animals respond poorly to existing vaccines which are optimized for the adult immune system. Our hypothesis is that protection against infection with EHV-1 can be induced in neonatal foals by a simple vaccination procedure that activates T-cell independent B-cell memory and antibody production. We performed one neonatal vaccination trial with our novel EHV-1 vaccine developed during the project and found proof-of-principle for our hypothesis. Neonatal foals received the vaccine at birth and were challenged by EHV-1 infection as weanlings. After EHV-1 challenge infection, vaccinated foals had significantly lower fever, reduced clinical signs, and higher EHV-1-specific antibody responses compared to the control group. The project takes an “outside-the-box” approach to stimulate a natural innate cytokine production pathway in neonates to overcome the challenges of EHV-1 vaccination early in life. The long-term goal of this proposed project is to prevent EHV-1 transmission and infection of foals early in life thereby reducing the population of latently infected horses and future EHV-1 outbreaks.
Development of a multiplex assay to determine antibodies to different glycoproteins of EHV-1

Laura B. Goodman, Heather Freer, Susanna Babasyan, Alicia Rollins, Gillian Perkins, Edward J. Dubovi, and Bettina Wagner

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Presenting Author: Laura B. Goodman

Development of rapid, sensitive and high-throughput assays for the assessment of immunity to equine herpesvirus type 1 (EHV-1) is important for vaccine development, clinical diagnostics and the evaluation of protective EHV-1 antibody levels in horses. The current gold standard is the serum neutralization (SN) test, which is time intensive and does not differentiate between EHV species. We have developed a new multiplex test based on two major EHV-1 antigens (glycoproteins C (gC) and gD). The antigens of interest were cloned and stably expressed in a mammalian expression system as IL-4 fusion proteins. Recombinant gC and gD antigens were then purified and coupled to fluorescent beads, which were used to quantify antigen-specific antibodies in horse serum on a Luminex platform. The assay was validated on serum samples from 58 horses. Validation samples included naturally EHV-1 infected, vaccinated or non-exposed horses with known SN titers ranging between <2 and 768. Multiplex-based antibody values were highly correlated with SN (R=0.87, p<0.0001). A group of 15 naïve horses were also monitored prior to and at monthly intervals following vaccination for one year to establish reference levels for protective immunity. In conclusion, the new multiplex EHV assay is an efficient alternative to SN testing.
Equine Herpesvirus Type 1 (EHV-1) is ubiquitous in horse populations, infecting up to 90% of horses worldwide. Foals often become infected at birth and, when the virus is reactivated, suffer from respiratory disease, neurological symptoms, or abortion. Currently, there are no approved neonatal EHV-1 vaccines, contributing to its worldwide prevalence. More information is needed about how foals’ immune systems respond to infection in order to develop an effective neonatal vaccine. It is well established that neonates of many species have immature immune systems. Neonatal horses, specifically, have recently been shown to have a decreased T helper cell Type 2 (Th2) response. This could imply an impaired B cell response to infection as Th2 cells play important roles in B cell activation. Surprisingly, antibody production of almost all immunoglobulin subclasses starts very early in the foal’s life, despite the impaired Th2 response. This suggests alternative mechanisms of neonatal B-cell activation and class switching in the absence of Th2 responses. This study seeks to characterize B-cell populations by analyzing the differences in total IgM, IgG1, and IgG4 expressing B-cells in 4 month, 1 year, 2 year old, and adult horses prior to EHV-1 infection. Subsequent infection studies (November 2013 and January 2014) will compare the total and EHV-1-specific B-cell response to infection.
Recombinant vaccines for equine herpesvirus type 1 (EHV-1) have the potential to block viral immune evasion while preserving full B- and T-cell antigenicity, which may lead to improved protection from clinical disease. Three putative immunomodulatory genes were deleted from neuropathogenic EHV-1 strain Ab4 and assessed for their contribution to immune modulation after infection of equine peripheral blood mononuclear cells (PBMC) in vitro. Protein levels of secreted equine interleukin-4 (IL-4), IL-10, IL-17, CCL2, IFN-g and IFN-a were simultaneously assessed; responsive cells were phenotyped by intracellular flow cytometry and quantitative RT-PCR. An ORF1/2 (UL56) gene deletion mutant reduced the induction of secreted type I and II interferons as well as pro- and anti-inflammatory cytokines compared to the parental strain, while intracellular IFN-g protein levels and IFN-g receptor transcription were increased. Strain-dependent regulation of T-cell transcription factors was also observed for UL56. Increases in TBet, GATA3, BCL6, and RORγ implicated effects on Th1, Th2, Tfh, and Th17 pathways respectively. Another mutant virus lacking the UL49.5 gene decreased type I interferon induction, while a virus lacking infected cell protein 0 (ICP0) had no effect on cytokine profiles in PBMC from previously exposed horses, except for a relatively small upregulation of CCL2. In naïve horses, however, the ICP0 deletion mutant induced lower levels of secreted IFN-g and IL-17 compared to wild-type virus. In conclusion, our data show that the EHV-1 homologs of ICP0, UL49.5, and UL56 have distinct immunoregulatory effects upon in vitro infection of horse PBMC, and Ab4 lacking UL56 is a strong vaccine candidate for prevention of neurologic EHV-1.
(#12) EHV-1-induced adhesion of platelets to equine endothelial cells

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Presenting Author: Wee Ming Yeo

Equid herpes virus-1 (EHV-1) is an important infectious cause of neonatal deaths, abortion and neurological disease in horses. Both abortion and neurological disease (the syndrome called equine herpes virus myeloencephalopathy or EHM) are caused by thrombosis of blood vessels supplying the placenta and spinal cord. The mechanisms underlying the development of thrombi in infected horses are unknown. Platelets are intimately involved in thrombus formation. Once activated, platelets adhere to blood vessels, form aggregates and amplify thrombin generation through exposure of the anionic membrane phospholipid, phosphatidylserine (PS). It is currently unknown if platelets are involved in the pathogenesis of thrombosis in horses with EHV-1 infection. We hypothesize that EHV-1 can bind to and activate equine platelets. Activated platelets could then become focus points of thrombi formation. Platelets incubated with the abortigenic of EHV-1 strain (RacL11) were activated after exposure to the virus for 10 minutes. When platelets were pretreated with RacL11 and introduced to CAECs grown in microfluidic channels at a 0.4 dyn/cm² flow rate, increased tethering and binding events were seen as compared to the control groups. These results indicate that EHV-1 can induce platelet activation and these activated platelets can then interact with equine endothelial cells, which could precipitate thrombosis.
Equine herpesvirus 1 (EHV1) infection of equine mesenchymal stem cells induces a pUL56-dependent downregulation of several cell surface markers

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Presenting Author: Christophe Claessen

Equine herpesvirus 1 (EHV1) is a ubiquitous alphaherpesvirus that can cause several symptoms including respiratory disease, abortion and central nervous disorders. Although it is known that EHV1 causes a cell-associated viremia, the exact identity of infected carrier cells in the blood remains a matter of debate. Recently, we found that most of the infected cells in the blood were positive for CD172a. Interestingly, mesenchymal stem cells (MSC), which can be found in the blood, have been shown to be CD172a positive also, at least in humans. The expression of CD172a on MSC of horses, which are being used therapeutically in various clinical settings, has not been evaluated before. Here, we report that equine peripheral blood-derived MSC (PB-MSC) are also positive for CD172a and susceptible to EHV1 infection. Interestingly, infection with EHV-1 resulted in a significant downregulation of the immunophenotypical cell surface markers CD29, CD105 and MHCI. In contrast, expression levels of CD172a, CD44 and CD90 remained constant. EHV1-mediated downregulation of cell surface MHCI has been described in other cell types and has recently been shown to depend on the viral pUL56 protein. We found that EHV1-mediated MHCI downregulation in equine PB-MSC is also pUL56-dependent. Importantly, and never reported before, we found that downregulation of CD29 and CD105 was also pUL56-dependent. In conclusion, we found that equine PB-MSC are susceptible to EHV1 infection, and that EHV1 infection results in a marked and pUL56-dependent downregulation of CD29, CD105 and MHCI. Our data indicated (i) a potential role of equine PB-MSC during EHV1 viremia, (ii) that pUL56 may target specific (clusters of) cellular proteins for downregulation, and (iii) that EHV1 infection may complicate correct identification and restrict therapeutic use of equine MSC.
Equine motor neuron disease (EMND) is a devastating disease of the horse with unknown etiology. EMND is marked by degenerative disorder of lower somatic motor neurons. The current research on putative pathway indicates that there is breakdown in the blood brain barrier (BBB) which might expose the central nervous system (CNS) to neurotoxins. However, the mechanism by which the integrity of the BBB is compromised is not fully understood. The BBB is composed of endothelial cells that protect brain from foreign substances. Multi-Drug Resistance (MDR-1) gene encoded the p-glycoproteins (P-gps) are important component of this barrier. The P-gps are primarily expressed in blood-tissue barriers responsible for intracellular and extracellular transport to protect the tissues. The epical surface of endothelial cells is marked by high concentration of P-gps. Mutations in the MDR-1 gene have been discovered in various dog breeds as well as in human and further studies are being carried out to investigate if these mutations affect the permeability of the BBB. Our studies showed that the Pgps are expressed in both EMND cases and control horses in different tissues. However, it is not clear if this expression is functional. We designed complementary studies to investigate the potential association between polymorphism in the MDR-1 gene and the risk of EMND.

Methods—An 895bp region of the MDR-1 gene was extracted from horse’s stabilized blood collected from EMND case positive and control horses. The amplified sequences were compared to the reference sequence from NCBI GenBank to identify single nucleotide polymorphism (SNP) that might associate with the risk of EMND.

Results—A total of eight SNP’s were detected in amplified MDR-1 gene among both EMND affected and control horses. The frequency of SNP’s in exon 26 was highest (≥ 70%) followed by exon 25 (50%) and exon 24 (40%). There were four SNP’s in exon 24 and two SNP’s each in exon 25 and 26 in all sampled population. All the detected SNP’s were synonymous among both EMND-cases and control horses.
**Conclusion**—Preliminary findings suggested that SNP’s in both cases and control are synonymous and the significance of this variation is still unclear. We hope that our ongoing studies could provide more insight into the role of these SNPs in the risk of EMND.

**Acknowledgements**—This research is partially supported by grant from Jack Lowe Funds at the College of Veterinary Medicine, Cornell University.

**Figure:** Data represent the frequency of single nucleotide polymorphism (SNP) in exon’s of 895 base pair (bp) amplified portion of horse MDR-1 gene in sampled population from EMND positive and control cases.
## APPENDIX D
2014 Harry M. Zweig Memorial Fund for Equine Research Awards

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<tr>
<td>Douglas Antczak</td>
<td>T-Cell Mediated Immunity and Vaccine Development in Horses (Year 2)</td>
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<tr>
<td>Thomas Divers</td>
<td>Etiology and Prevention of Equine Serum Hepatitis (Theiler’s Disease) (Year 2)</td>
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<tr>
<td>Bettina Wagner</td>
<td>Innate Immune Mechanisms and T-Cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses (Year 2)</td>
</tr>
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|                                  | Sub-Total: $205,499 |

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<tr>
<th>NEW/Renewal</th>
<th>ANNUAL AWARD</th>
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<tbody>
<tr>
<td>Dorothy Ainsworth</td>
<td>Fine Mapping of Candidate Genes Contributing to Equine Left Recurrent Laryngeal Neuropathy (RLN) Phase II (1 Year award)</td>
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<tr>
<td>Lisa Fortier</td>
<td>Cellular Biomarkers of Early Cartilage Injury Measured with Multiphoton Imaging (1 Year award)</td>
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<tr>
<td>Robert Gilbert</td>
<td>Effect of Early Pregnancy on Function of the Equine Corpus Luteum (1 Year award)</td>
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<tr>
<td>Alan Nixon</td>
<td>Evaluation of Lubricin as a New Biotherapeutic for Equine Joint Disease (2 Year award)</td>
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<tr>
<td>Rolfe Radcliffe</td>
<td>En Bloc Removal of Intravascular Thrombi via an Extracorporeal Bypass Circuit in Experimentally Induced Jugular Thrombosis in Horses (1 Year award)</td>
</tr>
<tr>
<td>Bettina Wagner</td>
<td>A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates (2 Year Award)</td>
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</tbody>
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|                                  | Sub-Total: $337,290 |
|                                  | TOTAL: $542,789 |
Soothing fraying nerves

The Harry M. Zweig Memorial Fund committee has selected equine surgeon Dr. Jon Cheetham as the first Zweig Research Scientist, a new position for junior faculty at Cornell’s College of Veterinary Medicine who show promise and productivity in equine research.

Awarded in December 2012, the yearlong position will fund Cheetham’s research into diagnosing and treating recurrent laryngeal paralysis, commonly known as roaring. Horses with this condition have developed weakened airway nerves and muscles and cannot breathe fully during exercise. Unfound and untreated, it can quickly end a horse’s career.

Cornell’s animal hospital is currently using the fruit of Cheetham’s past roaring research to help patients. Clinics now use the trans-esophageal ultrasound, a technique Cheetham developed to evaluate the geometry of horse’s airway muscles during rest and exercise. This technique allows clinicians to find roaring in young horses early, giving them a chance to recover.

Now Cheetham has turned his focus to improving the odds for that chance. His current project focuses on reimagining the nerve graft, a technique that takes a healthy nerve from neck muscles and puts it onto a damaged nerve to stimulate neglected muscles and help them recover.

“Nerve grafting has been around awhile, but it’s slow,” said Cheetham. “Currently you directly implant a healthy nerve into atrophied muscle, but only part of the muscle heals and it takes a long time. We’re working to develop a new, faster, better nerve graft method.”

With strong preexisting data supporting the concept, Cheetham will use his time as Zweig Research Scientist to develop a new method of enhancing nerve grafts. He has started pioneering research into extracellular matrix scaffolds, an area that has not been well explored.

“Our goal is to develop a way to manipulate the micro environment at a nerve repair site to promote healing,” said Cheetham. “I’m delighted to have this opportunity from the Zweig Fund to help expand healing options in horses suffering from roaring.”
Please welcome
Dr. Joel Baines as the College’s
Associate Dean for Research and Graduate Education.

With the title comes new responsibilities for the James Law Professor of Virology. As the associate dean, he oversees the doctoral program at the College of Veterinary Medicine; the Veterinary Investigators’ Program for veterinary students seeking research experiences; and several advanced training programs for clinicians, including the Clinical Fellows Program. He also directs efforts to help scientists secure external funding for research.

His most ambitious goal, though, has the potential to unite the worlds of basic and applied science in a manner that will advance the health and well-being of animals and people.

“The College is in a unique position to conduct research that benefits all species,” said Baines, who is leading an effort to strengthen the clinical research programs at the College. “We are at the beginning of a new era in veterinary medicine, an era in which disease mechanisms can be used to explain the physical manifestations of the condition. This level of understanding can lead to novel therapies.”

Baines, a Professor in the Department of Microbiology and Immunology at the College, joined the department in 1993. He received a BS in Microbiology from Kansas State University in 1979 and a VMD from the University of Pennsylvania in 1983. He then received a PhD from Cornell University in 1988, where he studied the molecular virology of feline coronaviruses.

He obtained postdoctoral training at the University of Chicago, where he studied the molecular virology of herpes simplex virus replication. In addition to his duties as associate dean, Dr. Baines will continue his research program with continual funding from the National Institutes of Health since 1995.

2013 Equine Seminar

On Sunday, August 25, at 2:00pm at Vernon Downs,
researchers from Cornell’s College of Veterinary Medicine will offer the following talks. Registration is at 1:30pm.

- “How to Protect Your Horse from Infectious Disease, at Home and at the Track,”
  Dr. Linda A. Mittel
- “Platelets and Herpes Virus Type-1 Infection,”
  Dr. Tracy Stokol
- “Diagnosis of Poor Performance in Racehorses”
  Dr. Jonathan Cheetham
- “Alternative Sources of Equine Mesenchymal Stem Cells”
  Dr. Gerlinde Van de Walle
Zweig Committee welcomes two new members

Dr. Janet Durso ’89

A veterinarian in Middletown, N.Y., Dr. Janet Durso joined the Zweig Committee in 2013. Her obsession with horses began when she was a little girl growing up in suburban Connecticut, where she fell in love with all things horses, working at local riding stables just to be near them.

After completing her undergraduate studies at Cornell University, she joined the polo team and remained in Ithaca to attend Cornell’s College of Veterinary Medicine and complete an internship in large animal surgery.

Durso has been an equine practitioner in the lower Hudson Valley since 1990, and started her own practice in 1994. Her husband is Ray Schnittker, a standardsbred trainer, driver, and owner whose outstanding career wins include Deweycheatumsnowe (2008 Hambletonian winner), One More Laugh (2010 Meadowlands Pace winner), and Check Me Out (2012 Zweig Trot winner as well as 2011 and 2012 Trotting Filly of the Year.) They have several standardsbred brood mares and stallion shares, as well as recent partnerships in some thoroughbred racehorses.

She worked as a part-time on-duty emergency veterinarian at the Meadowlands for 11 years and ran a bustling pleasure horse practice for many years, but has recently concentrated her practice on the family’s large racing stable. She is a member of the board of directors of the Goshen Historic Track and still finds time to play arena polo at Gardnertown Farms in Newburgh, N.Y.

Dr. Gabriel Cook ’92

Dr. Gabriel Cook joined the Zweig Committee in 2013. He graduated from Vassar College in 1986 and Cornell University College of Veterinary Medicine in 1992, then took an internship at the Rood and Riddle Equine Hospital in Lexington, Ky.

He completed a large animal residency at North Carolina State University in 1996 and subsequently served as a staff surgeon at a private practice in the San Francisco Bay area for two years. He is presently a partner at New England Equine Practice in Patterson, N.Y., where he has worked since 1998.

Dr. Cook’s clinical interests include medical and surgical lameness diagnosis and management; abdominal surgery; arthroscopy; laparoscopy; and critical care.
Dr. F. Richard Lesser ’81 has stepped down from the Harry M. Zweig Memorial Fund for Equine Research Committee to pursue a vocation in ordained ministry. Joining in 2004, he was a major proponent of the Zweig Fund’s mission to support research advancing the well-being of race horses. He offered expertise, heartfelt discussion, and sound judgment in helping administer the fund.

After a successful career as an equine veterinarian, Lesser retired in Fall 2012. He and his wife, Marilyn Schmidt ’78, founded the Equine Clinic at Oaken Croft in Ravena, NY. She died suddenly in 2005, and Lesser raised their three children on the family farm. In 2013 he was given the Lifetime Achievement Award by the Capital District Hunter Jumper Council, an award last presented in 2006 to Marilyn.

Lesser is dedicated to the betterment of equine practice. While managing an ambulatory and in-house equine clinic employing many veterinarians, interns, and support staff, he has also served as speaker, teacher, and advisor for equine and veterinary practices around the country. For years he engaged in the American Association of Equine Practitioners, serving on several committees and chairing the National Commission on Veterinary Economics Task Force and the Task Force on Equine Veterinary Technicians.

He spoke on equine issues to people around the country, taught equine health at SUNY Cobleskill, and chaired Veterinary Management Group VII, a group of 21 veterinary practices dedicated to bettering equine practice. He served as co-facilitator of Veterinary Management Group XX, a group of academic equine hospitals, including Cornell, dedicated to maximizing university clinics’ missions.

In May 2013 Lesser earned a Master in Divinity degree and will enter the Blessed John XXIII National Seminary as a student this Fall. We wish him well on his latest journey!
Many members of the Harry M. Zweig Fund Committee enjoy equine companions at home as well as work. This photo series runs over several Zweig News Capsule issues featuring Committee members with their horses.

Left:

Mr. Paul Kelly with “You Want Me,” a two-year-old Sire Stakes trotting filly champion for New York State in 2012.
Paternal genes make placentas

The mule has served humankind as a beast of burden from the time of Aristotle to the present day in the developing world. This hybrid between a male donkey and female horse and its lesser known relative, the hinny (a cross between a stallion and a female donkey), have made important contributions to our understanding of biology, particularly genetics.

Horsemen have long contended that mules and hinnies are distinct creatures, suggesting that their inheritance may not be equal—even though both inherit half their genes from horse and donkey parents. This presents a genetic conundrum.

The Baker Institute at Cornell’s College of Veterinary Medicine has used mules and hinnies in research since the 1980s. Most recently, Baker Institute scientists used mule and hinny tissues to examine gene expression in the placenta.

For this work, Dr. Douglas Antczak and his colleagues partnered with Drs. Andy Clark and Xu Wang of the Department of Molecular Biology and Genetics at Cornell to apply cutting-edge gene sequencing techniques to placenta samples from mules and hinnies. This approach enabled scientists to identify whether particular genes were expressed only from the father’s or mother’s chromosomes, or from both.

The study produced the most definitive evidence yet that there is a division of labor between genes of the mother and father in producing normal mammalian offspring. Examination of the so-called imprinted genes, a small subset of genes expressed only from the mother’s or father’s chromosomes, revealed that paternally expressed imprinted genes predominate in placental tissue. Such an outcome had been predicted 25 years earlier from studies of mouse embryos, but never verified experimentally.

This means that genes from its donkey father dominate the placenta of the mule, while in the hinny placenta the imprinted genes of the horse father are expressed. Because the imprinted genes of horses and donkeys carry distinct DNA sequences, this confirms that the horsemen’s folk adage is true—mules and hinnies are indeed genetically different!

The research also has implications for human health, where imprinted genes have been shown to be essential for normal fetal and placental growth and development.
Horse health tips

Dr. Linda Mittel, senior extension associate at the Animal Health Diagnostic Center, offers tips and good practices for equine biosecurity at home, boarding facilities, show grounds, and the track.

1) **Vaccinate.** Common available vaccines can protect against rabies, strangles, influenza, tetanus, and more.

2) **Don’t submerge** the ends of water hoses in water buckets. The outside of hoses often harbor disease causing bacteria or viruses. Keep them out of the water. Clean water troughs routinely.

3) **Don’t share or swap** buckets of water or food or hay nets. Other horses that use it may harbor pathogens.

4) **Don’t share tack** or equipment in the barn with different horses. Clean bits after use.

5) **Use separate grooming tools** for each horse.

6) **Use specially designated pitch forks** and wheelbarrows for animals that are sick in the barn. Colored duct tape can be used to color code items.

7) **Use foot baths** and ask visitors to do the same.

8) **Isolate / quarantine sick horses.** Have a stall or area designated as a quarantine stall prior to needing it.

9) **Decrease traffic** (people, horses, and other animals) from other barns.

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2013 Equine Seminar

Cornell University College of Veterinary Medicine faculty presented a seminar on advances in equine health practices and procedures for horse breeders, owners, and trainers on August 25, 2013 at Vernon Downs in Vernon, N.Y. The seminar covered infectious diseases, platelets and equine herpes virus type-1 infections, alternative sources of equine stem cells, diagnosis of poor performance in racehorses, and the importance of hepatitis virus to equine health. Question and answer opportunities followed. The event was sponsored by the Agriculture and N.Y. State Horse Breeding Development Fund and hosted by Harness Horse Breeders of N.Y.
The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry M. Zweig, a distinguished veterinarian, and his numerous contributions to the state’s equine industry. In 1979, by amendment to the pari-mutuel revenue laws, the New York State legislature created the fund to promote equine research at the College of Veterinary Medicine, Cornell University. The Harry M. Zweig Committee is established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians.

CORNELL UNIVERSITY
COLLEGE OF VETERINARY MEDICINE
2014 HARRY M. ZWEIG MEMORIAL FUND COMMITTEE

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Sr. Vice President, Operations
Blue Chip Farms, Inc.
Wallkill, N.Y.

Gabriel Cook, DVM
New England Equine Practice
Patterson, N.Y.

Janet Durso, DVM
Middletown, N.Y.

Paul Kelley
Kelley Racing Stable, LLC
Gansevoort, N.Y.

Michael I. Kotlikoff, VMD, PhD
Austin O. Hoey Dean of Veterinary Medicine
Cornell University College of Veterinary Medicine, Ithaca, N.Y.

Paul C. Mountan, DVM
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Robert Williams
Acting Executive Director
New York State Gaming Commission
Schenectady, N.Y.

William Wilmot, DVM
NYS Thoroughbred Breeding & Development Fund Corp. Saratoga Springs, N.Y.

Anna Zweig
Middlebrook Farms
Nassau, N.Y.

Brian Zweig
Rensselaer, N.Y.

Robert Williams was named Acting Executive Director of the New York State Gaming Commission by Governor Andrew M. Cuomo in January 2013. He most recently served as Acting Director of the New York Lottery. He also serves as Chair of the Franchise Oversight Board, providing oversight of the N.Y. Racing Association, Inc.’s finances. Williams served in 1996 as Counsel to the N.Y. State Task Force on Casino Gambling and as the 2005 to 2007 Executive Director of the New York State Committee on the Future of Racing. More info on Mr. Williams and other Zweig members: vet.cornell.edu/Zweig/Members.cfm

Zweig Committee retrospectives

For more than 30 years a diverse collection of people has dedicated time and energy to the Harry M. Zweig Memorial Fund Committee. The Zweig News Capsule will run an ongoing series of anonymous retrospectives like the notes below from current and former members. Contact lam78@cornell.edu to submit.

“We often discussed whether the research projects funded were practical and produced results that could be transferred directly and quickly to help meet industry needs. The types of research projects that did so quickly became the bread and butter of submitted proposals. Our accomplishments included getting more funding and earning greater respect for Cornell’s veterinary college.”

“In attending my first Zweig Fund Committee meeting, I was struck by the degree of passion all committee members had for equine science and the equine industry in general. The group is an eclectic one, with different experiences and expertise. As a “newbie,” I was content to listen and learn. It was invaluable, and I look forward to this year’s session with great enthusiasm.”

“How fortunate the equine community is having the great team of researchers at Cornell. Dr. Zweig certainly had great insight to develop this funding for equine research. The Zweig Fund has helped retain faculty members with diverse interests in many different curricula, which seem to complement each other; they truly are the cutting edge. Every member brings a different perspective to the table when deciding on allocation of funding. The scientific reviews are very helpful, and I am overwhelmed at all of the knowledge. It is a steep learning curve for a few years to understand the review process. The reward is seeing some of the results from the studies that were previously funded. Dr. [Rick] Lesser [a former member] will be missed; he contributed a tremendous amount of insight and experience to the review process.”
Winner’s Circle

“Mistery Woman,” winner of this year’s Zweig Trot held at Vernon Downs on Sunday, August 25, 2013.