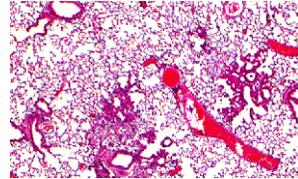


Feature Article

Beth Callan
VMD



FACULTY GATHER AT ANNUAL RETREAT



RESEARCH RESOURCES PRESENTED AT RETREAT



PRESENTATIONS AT RETREAT – PAGES 4-5

NEWSLETTER



Gina Milani, CVT, Emergency Service, Dr. Beth Callan, and Penn Animal Blood Bank Manager, Kym Marryott, CVT, with donor dogs, Fred and Memphis

It's in the blood

Beth Callan is a professor of medicine (clinician-educator track) in the Department of Clinical Studies-Philadelphia and medical director of the Penn Animal Blood Bank. Beth received a B.S. in Chemistry from Chestnut Hill College in 1984 and a V.M.D. from Penn Vet in 1988. She then completed a rotating internship, residency in internal medicine, and fellowship in hematology/transfusion medicine, all at Penn Vet, and has since remained affiliated.

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The 2015 **Zoetis Award for Veterinary Research Excellence** was awarded to **Dr. P Jeremy Wang**, Department of Biomedical Sciences, at the Annual Faculty Research Retreat on June 19th. Dr. Wang has a publication appearing in *EMBO* entitled "TEX11 is mutated in infertile men with azoospermia and regulates genome-wide recombination rates in mouse", 2105, *EMBO Mol Med.* 2015 Jul 1. pii: e201404967, *Epub ahead of print.*



Dr. Callan, continued from page 1

Beth's current clinical research interests are centered on red blood cell (RBC) storage and gene therapy for canine hemophilia.

Duration of RBC storage: Is old blood bad?

The shelf-life of stored RBCs is typically 42 and 35 days for human and canine RBCs, respectively. Given that blood is a precious and limited resource, both human and veterinary blood banks typically dispense the oldest RBC units first to reduce wastage. However, accumulating evidence suggests that transfusion of RBCs stored for 14 days or longer increases rates of infection, thrombosis, morbidity, and mortality in hospitalized human patients. During storage *in vitro*, RBCs undergo cumulative biochemical and biomechanical changes that reduce their recovery *in vivo*. After transfusion, such storage-damaged RBCs are quickly cleared from the circulation by reticuloendothelial macrophages and the consequent catabolism of hemoglobin leads to a rapid release of iron. If iron is returned to plasma at a pace that exceeds the rate of transferrin-mediated iron uptake, circulating non-transferrin-bound iron (NTBI) levels increase. As NTBI participates in redox reactions that promote oxidative damage, cytotoxicity, and endothelial activation, determining the time frame in which stored RBCs can be safely utilized for transfusions is critical for optimal outcome.

In collaboration with Dr. Eldad Hod from Columbia University, Dr. Callan investigated the effect of duration of RBC storage on transfusion-associated inflammation in healthy dogs receiving autologous RBC units stored for 7 (fresh) and 28 (old) days. Administration of old, but not fresh, RBC units was associated with a pro-inflammatory cytokine response, exemplified by monocyte chemoattractant protein-1, and accompanied by increased neutrophil counts and decreased platelet counts (Figure 1).¹ In addition, old RBC transfusions were associated with decreased post-transfusion RBC recovery, increased serum bilirubin (evidence of extravascular hemolysis), and increased NTBI at two and six hours post-transfusion. Together with the finding that transfusion with old RBC units was a negative risk factor for survival in dogs with immune-mediated hemolytic anemia (IMHA),² Drs. Callan, Hod, and Thawley (Penn Vet), are conducting a randomized, blinded clinical trial in which client-owned dogs with primary IMHA receive fresh (stored <7 days) or old (stored 21-28 days) RBCs. *continued on page 3*

Publications



Yu, Q., Carbone CJ, Katlinskaya YV, Zheng H, Zheng K, Luo M, Wang PJ, Greenberg RA & Fuchs SY (2015) Type I

Interferon Controls Propagation of Long Interspersed Element -1. *J Biol Chem* 290 (16) 10191-10199.



T. Bale (2015) Epigenetic and Trans-generational Reprogramming of Brain Development. *Nat Rev Neurosci*. 16(6): 332-44



Gaub, B.M., Berry, M.H., Holt, A.E., Reiner, A., Kienzler, M.A., Dolgova, N., Nikonov, S., Aguirre, G.D., Beltran,

W.A., Flannery, J.G., Isacoff, E.Y. Restoration of visual function by expression of a light-gated mammalian ion channel in retinal ganglion cells or ON-bipolar cells. (2014) *PNAS USA* 111 (51) E5574-83.



F Zhang, D Patel, K Colavita, I Rodionova, B Buckley, David Scott, A Kumar, SA Shabalina, S Saha, M Chernov, A Osterman, & Anna Kashina. (2015)

Arginylation Regulates Purine Biosynthesis by Facilitating the Biological Activity of Phosphorybosyl Pyrophosphate Synthase. *Nat. Commun.* 6:7517, in press.

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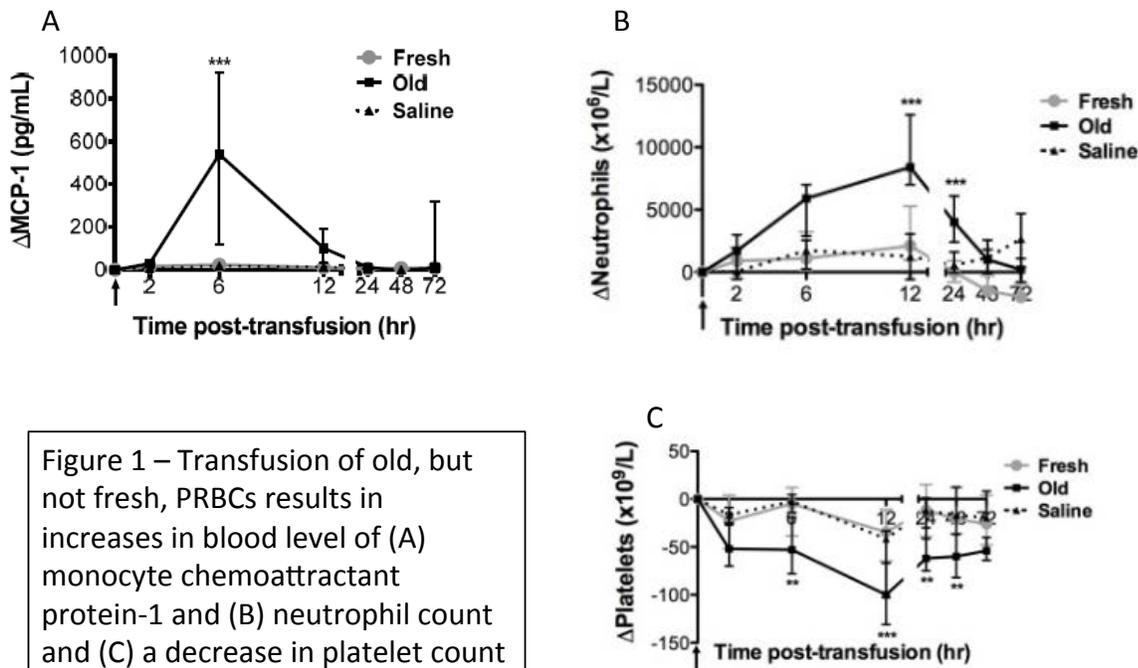


Figure 1 – Transfusion of old, but not fresh, PRBCs results in increases in blood level of (A) monocyte chemoattractant protein-1 and (B) neutrophil count and (C) a decrease in platelet count (median \pm interquartile range).

If transfusion of “older” RBCs to dogs with IMHA is associated with an increased inflammatory response, increased morbidity and/or mortality, it will support changing current transfusion practices to include the use of “fresh” RBCs to transfuse anemic canine patients. As such, this study will have a significant impact on canine health and veterinary blood banks. However, as discarding older RBCs could lead to a shortage of RBC units, statistically significant findings are clearly needed to warrant such a change.

Gene therapy: Hope for dogs with hemophilia

Hemophilia A (HA) and hemophilia B (HB) are hereditary bleeding disorders caused by a deficiency in the blood coagulation factor VIII (FVIII) and factor IX (FIX), respectively. These coagulopathies occur in both humans and dogs and in their most severe form result in spontaneous bleeding, most commonly into joints, hematoma formation and potentially life-threatening hemorrhage following trauma or surgery. Humans with severe HA or HB require

prophylactic administration of recombinant human FVIII or FIX protein several times weekly to control bleeding. Research dogs with HA and HB have been used as models to evaluate novel therapies for human HA and HB. Gene transfer using an adeno-associated viral (AAV) vector to deliver the genes for canine FVIII (cFVIII) and FIX to dogs has been successfully performed in more than 70 research dogs and such studies reveal that a 2-3% increase in FVIII and FIX activity reduces bleeding episodes by >90%. Long-term follow-up (> 9 years) has documented the efficacy and safety of gene therapy using AAV vectors to ameliorate bleeding tendencies in research dogs.

In collaboration with Drs. Katherine High and Valder Arruda from Children’s Hospital of Philadelphia (CHOP) and Dr. Mark Haskins (Penn Vet), Dr. Callan has adopted this technique to treat two privately owned dogs with severe HA (plasma FVIII activity < 1%) using an AAV8-cFVIII vector administered as a

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ANNUAL FACULTY RESEARCH RETREAT at PENN VET

On **June 19, 2015**, 115 faculty, residents, postdocs, students and staff gathered for the **Annual Faculty Research Retreat** at New Bolton Center. **Phillip Scott**, Vice Dean for Research and Academic Resources convened the conference. James M Wilson, M.D., Ph.D., delivered the Robert R. Marshak Lecture entitled “Gene Therapy for Animal and Human Health”. Penn Vet’s research resources were presented in talks by **Tom Parsons**, V.M.D., Ph.D., Swine Teaching & Research Center—A Pig’s Eye View of Animal Welfare; **William Beltran**, D.V.M., Ph.D., Targeting Gene Therapy for X-linked Retinitis Pigmentosa to Patient-relevant Stages of Disease; **J.Oriol Sunyer**, Ph.D., Fish Immunology —Exploring the Evolution of Immunity; **Michael Povelones**, Ph.D., Insectary: Exploring Mosquito -Pathogen Interactions; **Charles Vite**, D.V.M., Ph.D., Referral Center for Animal Models of Genetic Disease; **Christopher Lengner**, Ph.D., Center for Animal Transgenesis and Germ Cell Research-Implementation of CRISPR/Cas9-Mediated Genome Editing; **Dorothy C. Brown**, MSCE, D.V.M., Veterinary Clinical Investigations Center—Making the Impossible Studies Possible; **Thomas Schaer**, V.M.D., Preclinical Research Services-Challenges and Opportunities. Four Penn Vet Cores were also presented: Comparative Pathology (**Amy Durham**,

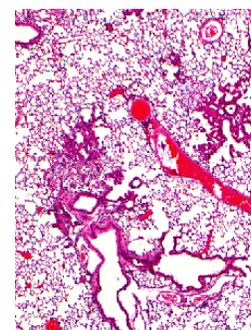
VMD, DACVP); Penn Vet Imaging (**Bruce Freedman**, PhD); Center for Host-Microbial Interactions Bioinformatics (**Dan Beiting**, PhD); and the Non-Invasive Whole Animal Imaging Facility (**Ellen Puré**, PhD).



Dean Joan Hendricks presented the Zoetis Award for Veterinary Research Excellence to P.Jeremy Wang, Ph.D., M.D.



Dr. James Wilson, Perelman School of Medicine, director of Penn’s Gene Therapy Program and the Orphan Disease Center at Perelman School of Medicine.



Dr. Dan Beiting of the Center for Host-Microbial Interactions.



Dr. Michael Povelones presented the ‘Insectary’ that explores mosquito-pathogen Interactions.



Dr. Amy Durham described Penn Vet’s Comparative Pathology Core



Dr. William Beltran reported on the Retinal Research Facility—Canine Models



Visiting Khorana Scholar, Shreya Nahata, attends the program



The Allam House at New Bolton Center—a beautiful setting for the annual faculty research retreat



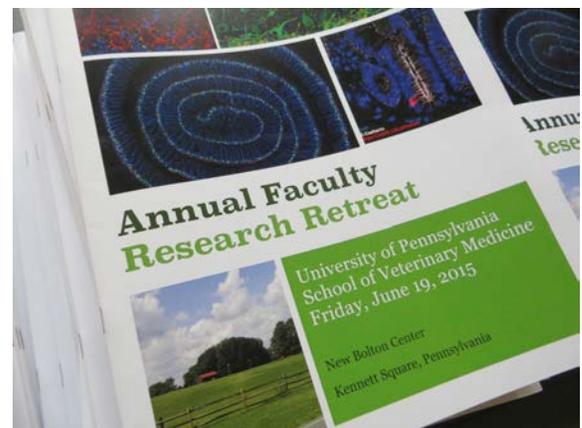
Dr. Oriol Sunyer gave his talk on the Penn Vet fish immunology facility and the evolution of immunity



Dr. Chris Lengner spoke about his research and the Center for Animal Transgenesis and Germ Cell Research



Participants enjoy posters and lunch



Dr. Callan continued from page 3

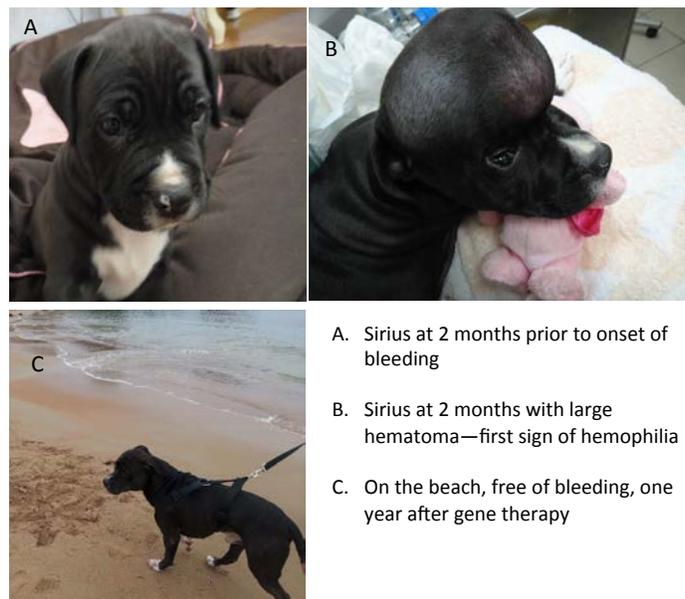
single peripheral venous injection. Both dogs had experienced 11 to 12 bleeding episodes that required transfusion support during the year prior to treatment (Figure 2). Following gene therapy there was a 5-fold increase in circulating FVIII, with sustained expression for at least 18 months post-treatment, and no signs of adverse events. The dramatic reduction in bleeding episodes in the two privately owned dogs has been life-changing (and potentially life-saving) for them.

Based on these promising results, the Penn Vet/CHOP collaborative group is undertaking a pilot study (funded in part by the Institute for Translational Medicine and Therapeutics) to evaluate a new variant of the AAV8-cFVIII vector in treating privately owned dogs with HA with severe bleeding tendencies. Like humans, dogs with hemophilia may develop antibodies that inhibit FVIII or FIX function following repeated plasma transfusions, making it more challenging to control bleeding. Notably, our ability to treat privately owned (outbred, as opposed to inbred research colony dogs) hemophilia dogs with different FVIII mutations provides a unique opportunity to gain further insights into the risk of immune responses in gene and protein therapy. Finally, FVIII and FIX promote coagulation by converting FX to factor Xa (FXa) to promote thrombin generation and fibrin clot formation. We are currently determining if infusion of a zymogen-like FXa variant that has a longer half-life and is less active than wild-type FXa (to prevent excessive coagulation) may be helpful in controlling bleeding in dogs with hemophilia, even in the presence of inhibitory antibodies. Dr. Callan predicts that such studies will greatly benefit canine hemophilia patients and are of direct translational importance to human patients as well.

Support for Dr. Callan’s research comes from the AKC Canine Health Foundation, the Companion Animal Research Fund (Department of Clinical Studies PHL), and the Institute for Translational Medicine and Therapeutics of the Perelman School of Medicine and the School of Veterinary Medicine at the University of Pennsylvania.

References:

1. Callan MB, Patel RT, Rux AH, Bandyopadhyay S, Sireci AN, ODonnell PA, Ruane T, Sikora T, Marryott K, Sachais BS, Hod EA. Transfusion of 28-day-old leucoreduced or non-leucoreduced stored red blood cells induces an inflammatory response in healthy dogs. *Vox Sanguinis* 2013;105:319–327.
2. Hann L, Brown DC, King LG, Callan MB. Effect of duration of packed red blood cell storage on morbidity and mortality in dogs after transfusion: 3,095 cases (2001–2010). *Journal of Veterinary Internal Medicine* 2014;28:1830–1837.



A. Sirius at 2 months prior to onset of bleeding
 B. Sirius at 2 months with large hematoma—first sign of hemophilia
 C. On the beach, free of bleeding, one year after gene therapy

Figure 2

RECENT AWARDS

(Direct Costs)

Montserrat Anguera
University Research Foundation
Faulty X-chromosome silencing as
significant contributor for female-bias
in autoimmunity
\$47,000 8/1/15 to 7/31/16

Christopher Lengner
University Research Foundation
Identifying markers for the prospective
isolation of reserve intestinal stem cells
\$50,000 8/1/15 to 7/31/16

Olena Jacenko
University Research Foundation
**Could a young niche rejuvenate
hematopoiesis**
\$50,000 8/1/15 to 7/31/16

Kendra Bence
Howard Hughes Medical Institute:
Chronobiology Program—
Investigating a novel link
between phosphatase
regulation of central GLP-1
activity and the circadian
control of feeding \$30,000
5/1/15-4/30/16

Jeremy Wang
NIH/U01HD084007
Targeting the piRNA pathway
and meiotic recombination for
male contraception
\$1,400,000 —5/01/2015-3/31/20

Oriol Sunyer
NIH R01 GM085207
Primordially conserved
principles governing mucosal
immune responses to
pathogens and microbiota
\$862,045 2/15/15—11/30/18

Leszek Kubin
NIH/R01 HL047600
Premotor control of upper
airway and REM sleep atonia
\$1,000,000 7/15/15—4/30/19

Carolina Lopez
NIH R21 AI109472
IL-10 producing neutrophils
during respiratory virus
infection
\$275,000 7/15/15-7/14/17

Jorge Alvarez
McCabe Foundation Pilot
Fund: The role of the
Hh pathway in regulating CNS
immunity. \$19,323
7/1/2015—6/30/2016

Jorge Alvarez
Multiple Sclerosis Society of Canada
Role of Non-Conventional CNS
Barriers During Homeostasis and
Neuro-inflammation.
\$278,000 1/1/15-12/31/17

Michael Povelones
McCabe Foundation Pilot
\$19,323 7/1/15—6/30/16

Lisa Murphy
USDA—Maintenance of Membership
Laboratory Requirements
\$50,000— 4/1/15-3/31/16

Ron Harty
Fox Chase Chemical Diversity Center
Development of small molecule
therapeutics against RNA viruses
\$84,374 3/1/15-2/29/16

Igor Brodsky
Burroughs Wellcome Fund
Investigators in the Pathogenesis of
Infectious Disease Award—Defining
the role of caspase-8 in the regulation
of anti-microbial host defense.
\$500,000 —7/1/15-6/30/20

James Marx
ASLAP Foundation Summer Fellowship
Program
\$5,000 —5/1/15-9/30/15

Louise (Southwood) Parente
North Carolina State U (Grayson
Jockey Club)
Flunixin or Firocoxib in postoperative
colic patients
\$12,958—5/1/2015-3/31/2017

Raymond Sweeney
Immune Solutions Ltd
Evaluation of an oral vaccine for Johne's
Disease in calves
\$97,760 5/1/2015-4/30/2016

Publications



Benitez AA, Panis M, Xue J, Shim JV, Varble A, Frick AL, López CB, Sachs D, and tenOever BR. (2015) In vivo RNAi screening identifies MDAs as a contributor to the cellular defense against influenza A virus. *Cell Reports*, online (June) doi: 10.1016/j.celrep.2015.05.032.



Srinivasan S, Guha M, Dong DW, Whelan KA, Ruthel G, Uchikado Y, Natsugoe S, Nakagawa H, Avadhani NG. (2015) Disruption of cytochrome c oxidase function induces the Warburg effect and metabolic reprogramming. *Oncogene*, doi: 10.1038/onc.227. [Epub ahead of print]



Glennie DN, Yeramilli VA, Beiting DP, Volk SW, Weaver CT, and Scott, P. (2015) Skin resident memory CD4+ T cells enhance protection against *Leishmania major* infection. *J Exp. Med.*, in press



Kopper J, Stewart S, Habecker P, Aitken M, Southwood LL*. Small Colon Stenosis Secondary to Ulcerative Colitis in Three Standardbred Foals. *Equine Vet Educ* 2015[Epub ahead of print] doi: 10.1111/eve.12273



Igor E. Brodsky, PhD is one of five early-career researchers at the **University of Pennsylvania** who has received [funding from the Burroughs Wellcome Fund \(BWF\) for their excellence in biomedical research](#), in topics including heart disease, sleep, and infectious diseases. Dr. Brodsky is an assistant professor in the Department of Pathobiology, **School of Veterinary Medicine**. He will focus on the activity of an enzyme called caspase-8, which plays a key role in how the immune system defends against invading microbes. His research could help identify therapeutic targets to either boost or tone down the immune system's response to infection or inflammation. (see *awards page 7*)



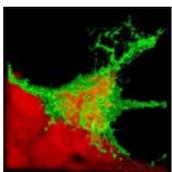
HONORS



In May, Pathobiology post-doctoral researcher, Dr. **Ana Mistic** spoke at the 4th Annual American Society For Microbiology conference on Methicillin-resistant *Staphylococci* in Animals: Veterinary and Public Health Implications. She spoke on antimicrobial resistance in zoonotic bacteria and foodborne pathogens. Her talk was entitled: The Finished Genome and Methylome for the Veterinary Pathogen, *Staphylococcus schleiferi*.



In April, **Ciara Gimblet**, (graduate student in the Scott Lab) gave an oral presentation at the **Woods Hole Immunoparasitology Meeting**, where she won an award for the **Best Presentation!** The title of her talk was: "*Leishmania major* induces transmissible alterations in the skin microbiome".



VMD/PhD student **Jonathan Madara** for winning the Perelman School of Medicine 2015 "Art in Science" competition. He won in the graduate student level for his image of "Ebola Virus-like Particle Budding". Jonathan's mentor is Dr **Bruce Freedman** and his collaborators were Dr. Ron Harty & Dr. Gordon Ruthel (PennVet Imaging Core)



Ralph Brinster, VMD, PhD, received an honorary doctor of laws degree in May from the University of Calgary. The highest academic honor is bestowed on individuals whose notable achievements and community service merit recognition. Ralph Brinster, one of the world's most accomplished veterinary scientists, received the honors at the Cumming School of Medicine and the faculties of law, veterinary medicine and graduate studies. A Richard King Mellon Professor of Reproductive Physiology at Penn Vet, he continues to perform cutting-edge research and is developing novel techniques for the culture and genetic modification of germline stem cells to restore fertility in male children undergoing cancer treatment.



Dr. **Meryl Littman** spoke at the **NY State Spring Veterinary Conference** on tickborne diseases on May 15-17 2015. She also spoke on Lyme disease at the ACVIM 33rd National Medical Forum, Indianapolis, in June 2015.

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Suggestions, requests, comments and story ideas may be directed to:

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