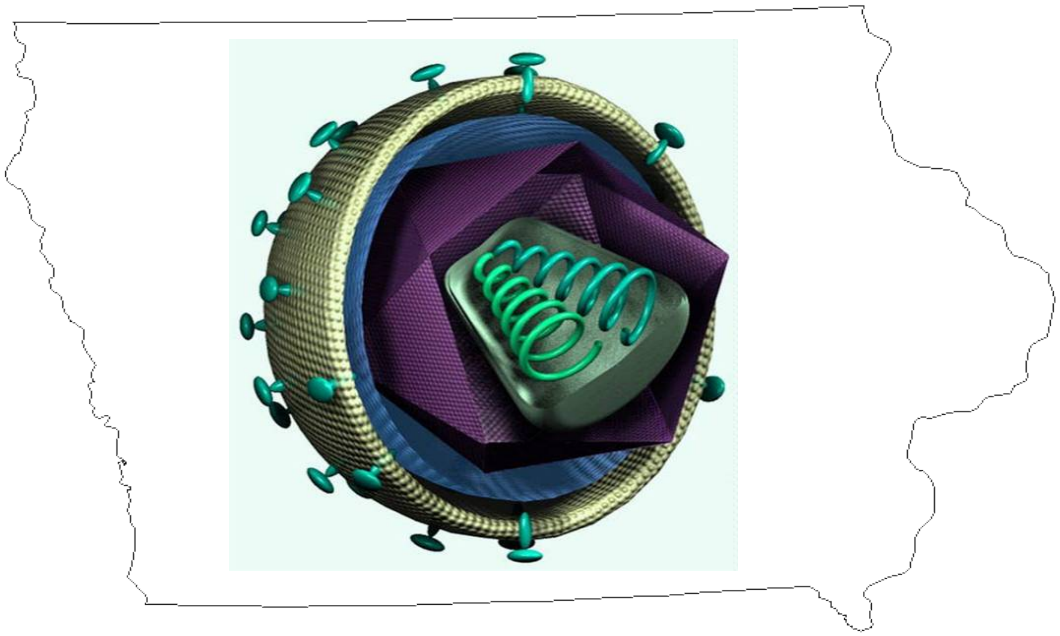
THE NINTH BIENNIAL

ALL-IOWA VIROLOGY SYMPOSIUM



MARCH 31 AND APRIL 1, 2017

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This symposium is dedicated to Prem S. Paul

The 2017 All Iowa Virology Symposium is dedicated to Prem S. Paul, D.V.M., Ph.D., to honor his many contributions to the Iowa virology community. Dr. Paul, a prominent animal virologist from Iowa State University, sadly passed away in September, 2016.

Dr. Paul was the veterinary medical officer at the USDA National Animal Disease Center in Ames, IA from 1978-1985, before joining the faculty of ISU where he served as director of graduate education (1991-1992), associate director of the Veterinary Medical Research Institute (1993-1999), assistant director of the Iowa Agricultural and Home Economics Experiment Station (1996-2000), associate dean for research and graduate studies for the College of Veterinary Medicine (1993-1999) and associate vice provost for research (2000-2001). In 2001, Dr. Paul accepted the position of dean of graduate studies and vice chancellor of research at the University of Nebraska–Lincoln in Lincoln, NE.

Prem S. Paul, D.V.M., Ph.D

Dr. Paul's scientific expertise is in animal virology. His early work focused on poultry viruses such Marek's disease virus and reticuloendotheliosis virus. At Iowa State University, his research focus on viral pathogenesis of respiratory and enteric diseases in swine resulted in significant contributions to animal health, including improved vaccines and diagnostic tests.

Dr. Paul was a member of the American Veterinary Medical Association, American Association for the Advancement of Science, American College of Veterinary Microbiologists, American Society for Virology, Conference of Research Workers in Animal Diseases and the American Association of Swine Veterinarians.

Dr. Paul published over 100 peer-reviewed research articles and 11 books, and held more than 20 U.S. and international patents.

All Iowa Virology Symposium

March 31 and April 1, 2017

PROGRAM

9:00-10:00	**Registration-**Packet Pick-up on 1st floor Continental Breakfast and beverages served Speakers upload presentations onto computers
9:55-10:00	Opening Remarks: Brad Blitvich
10:00-10:05	Introduction of Plenary Speaker: Rich Roller
10:05-11:00	**Plenary Speaker: Rob Kalejta, PhD,** University of Wisconsin-Madison How Human Cytomegalovirus Regulates Viral Transcription and Brain Cancer
Session 1:	**Immune Response to Viral Infections**
	Convener: Susan Carpenter
11:00-11:15	Stress Granule Modulation by Mammalian Orthoreovirus Factories **Promisree Choudhury**, Cathy L. Miller
11:15-11:30	PARP-dependent ADP-ribosylation independently enhances the IFN response and represses coronavirus replication **Anthony R. Fehr**, Gytis Jankevicius, Craig Fett, Ivan Ahel, Stanley Perlman
11:30-11:45	The Effect of Macrophage M2 Polarization on Ebola Virus Infection **Kai Rogers,** Ayithan Natarajan, Wendy Maury
11:45-12:00	The MHV packaging signal promotes virulence and prevents a robust interferon response during infection. **Jeremiah Athmer**, Anthony Fehr, Mathew Grunewal, Stanley Perlman
12:00-1:30	Lunch, Ballroom
Session 2:	**Virus Replication and Gene Expression**
	Convener: W. Allen Miller

1:30-1:45	The 3' Untranslated Region of Maize Chlorotic Mottle Virus Contains a New Type of Cap-independent Translation Enhancer **Elizabeth J. Carino**, W. Allen Miller, Kay Scheets, Keisuke Komoda, Sung Ki Cho, Jelena Kraft
1:45-2:00	Insights into Epstein-Barr virus biology through the study of RNA structure **Walter Moss**
2:00-2:15	Long-distance interactions in the xrRNA structure are required for exonucleolytic generation of the 3' noncoding subgenomic RNA of barley yellow dwarf virus. **Keisuke Komoda**, W. Allen Miller
2:15-2:30	Identification of a conserved coiled-coil-like motif in retroviral Rev-like proteins Chijioke N Umunnakwe, Drena Dobbs, Karin S. Dorman, **Susan Carpenter**
2:30-2:45	Foxtail Mosaic Virus Based Vectors for Gene Silencing and Gene Expression in Maize **Yu Mei**, Chunquan Zhang, Bliss M. Kernodle, Mingsheng Qi, Katherine L. Quandt, John H. Hill, Steven A. Whitham
2:45-3:00	Pathogenesis and Cross-protection of U.S. PEDVs **Jianqiang Zhang**, Qi Chen, Joseph Thomas, Phillip Gauger
3:00-3:30	**Break**
Session 3:	**Virus-Host Interactions** Convener: Wendy Maury
3:30-3:45	Cellular stress response to varicella-zoster virus infection of human skin includes both elevated interleukin-6 transcription and robust autophagy John E. Carpenter, **Erin M. Buckingham**, Wallen Jackson, Keith W. Jarosinski, Kevin Knudtson, Charles Grose
3:45-4:00	Dynamics of semen exosome-mediated HIV inhibition **Jennifer L. Welch**, Chioma M. Okeoma
4:00-4:15	Introduction of a fluorescent competent tetracysteine tag within mammalian orthoreovirus non-structural protein μNS for visualization of viral factory dynamics

	Luke D. Bussiere, Promisree Choudhury, Bryan Bellaire, Cathy L. Miller
4:15-4:30	Ebolavirus Glycoprotein Residue 95 Alters Endosomal Requirements for Virus Entry Nicholas J. Lennemann, **Jacob Dillard**, Ashley Cooney, Grace Shaack, Robert A. Davey, Wendy Maury
4:30-4:45	Extragenic Suppression of a Mutation in Herpes Simplex Virus Type-1 (HSV-1) that Affects Lamina Disruption and Nuclear Egress **Amber Vu**, Chelsea Poyzer, Richard Roller
4:45-5:00	Interaction of HIV-1 Env with Membrane Lipids Maintains the Trimer in a Closed Functional Conformation **Jacklyn Johnson**, Hamid Salimi, Michael Zhang, Manuel Flores, Yunxia O'Malley, Jon Houtman, Patrick Schlievert, Hillel Haim
5:00-5:15	Set up posters
5:15-7:00	Poster session, 2nd floor Hors d'oeuvres and bar
7:00-8:00	Banquet
8:00-8:15	Prem Paul dedication and Introduction of Plenary Speaker: Susan Carpenter
8:15-9:15	**Plenary Speaker: X.J. Meng, PhD,** Virginia Tech The ever-expanding host range and cross-species infection of hepatitis E virus
Saturday April 1	
8:30-9:00	Refreshments
9:00-9:05	Introduction of Plenary Speaker: Cathy Miller
9:05-10:00	**Plenary Speaker: Nihal Altan-Bonnet, PhD,** NIH Viral propagation in vesicles: pooped out or pathogenic
10:00-10:15	Break
Session 4:	**Vaccines and Therapeutics**
	Convener: Chioma Okeoma

10:15-10:30	Equine influenza elicits multiple broadly neutralizing antibodies to hemagglutinin **Sarah A. Wiechert**, Jessie Trujillo, Richard Webby, Sandhya Bangaru, James Crowe, Brian Lee, Brett A. Sponseller, David Verhoeven
10.30-10.45	A piggyBac transposon/adenoviral viral-based hybrid vector system for improved in vivo CFTR delivery **Ashley L. Cooney**, Ni Li, Brajesh Singh, David A. Stoltz, Paul B. McCray Jr., Patrick L. Sinn
10:45-11:00	Antiviral strategies against human respiratory syncytial virus (RSV) in perinatal and newborn lambs **Mark R. Ackermann**
11:00-11:15	Determination of the immunodominant regions of Senecavirus A-VP1 by ELISA epitope mapping Houston ER, Gimenez-Lirola LG, Sinha A, Shen H,Villarino NF, **Piñeyro PE**
11:15-11:30	Nanovaccine-mediated immune protection against influenza A virus **Zeb R. Zacharias**, Kathleen A. Ross, Jonathan T. Goodman, Balaji Narasimhan, Thomas J. Waldschmidt, Kevin L. Legge
11:30-11:45	Closing Remarks: Cathy Miller

Oral

Abstracts

1.	**Stress Granule Modulation by Mammalian Orthoreovirus Factories**
	Promisree Choudhury and Cathy L. Miller Department of Veterinary Microbiology and Preventive Medicine, Iowa State University
	Phosphorylation of eIF2α induces formation of discrete cytoplasmic inclusions called stress granules (SG). SGs are comprised of translationaly silenced mRNAs, translation initiation factors, ribosomal proteins, and SG effector proteins such as TIA-1, TIAR, G3BP1 and G3BP2. Emerging evidence suggests that SGs are the site of activation of proteins critical in the innate immune response to virus infection, such as PKR and RIG-I. MRV entry into cells induces SG formation, however, as virus gene products are synthesized, SGs are disrupted even in the presence of phosphorylated eIF2α, suggesting MRV is able to disrupt virus-induced SGs in a manner dependent on new viral protein translation. In this work, we sought to determine the mechanism of MRV mediated SG modulation. Our results demonstrate that in many infected cells, SG effector proteins display localization peripheral to virus encoded structures termed viral factories (VFs) that are formed during MRV infection and facilitate viral transcription, translation, replication and assembly. In transfected cells, localization of SG proteins around VF-like structures (VFLs), formed solely by MRV μNS, was not detected. However, addition of non-structural protein σNS resulted in strong recruitment of G3BP1, G3BP2, Caprin1 and USP10 to VFLs. Moreover, σNS associated with G3BP1 in an immunoprecipitated protein complex either in the presence or absence of μNS. SG formation was not necessary for localization of SG proteins to VFLs, but VFLs containing σNS were able to inhibit SGs induced by the cellular stressor sodium arsenite, suggesting σNS dual association with G3BP1 and μNS may prevent SG formation during MRV infection. Localization of G3BP1 to VFLs was dependent on RNA binding domains of both σNS and G3BP1, as well as eIF2α phosphorylation and PKR activation. Moreover, replication of MRV was enhanced in cells lacking G3BP1, G3BP2 or both G3BP1/2. Taken together, our results implicate a key role of MRV VFs in SG modulation, mediated via the viral proteins μNS and σNS. These data suggest MRV factories may play an active role in disruption of the innate immune response to infection.

2.	**PARP-dependent ADP-ribosylation independently enhances the IFN response and represses coronavirus replication**
	Anthony R. Fehr[1], Gytis Jankevicius[2], Craig Fett[1], Ivan Ahel[2] and Stanley Perlman[1*] [1]Department of Microbiology, University of Iowa, Iowa City, IA 52242; [2]Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK.
	ADP-ribosylation mediated by ADP-ribosyl transferases (PARPs) is a common post-translational modification (PTM) that may have antiviral properties and impact innate immunity. However, this has not been demonstrated during a natural virus infection. To regulate this activity, macrodomain proteins enzymatically remove ADP-ribose from protein targets. All members of the Coronavirinae, a subfamily of positive sense RNA viruses, contain a highly conserved macrodomain within non-structural protein 3 (nsp3) that can remove ADP-ribose from protein *in vitro*. Mutation of the CoV macrodomain active site greatly reduced its deADP-ribosylating activity. Both SARS-CoV and MHV recombinant viruses with these mutations were highly attenuated, had reduced viral loads in infected mice, and elicited an enhanced interferon (IFN) response that contributed to the protection of infected mice. Here, we utilized JHMV infection of bone-marrow derived macrophages (BMDMs) to further understand macrodomain function as mutant virus (N1347A) had reduced virus replication and induced a strong increase in IFN production in these cells. MAVS was required for the N1347A-mediated IFN induction, as the IFN response was blunted following infection of $MAVS^{-/-}$ cells with N1347A. However, N1347A still replicated poorly in $MAVS^{-/-}$ cells, indicating that the macrodomain promotes replication independently of its ability to suppress the IFN response. Finally, we found that both IFN levels and virus replication following infection with N1347A were reversed in the presence of PARP inhibitors, demonstrating a clear role for ADP-ribosylation in enhancing the immune response and repressing CoV replication. In conclusion, we have established ADP-ribosylation as an antiviral PTM that can control CoV replication and pathogenesis.

3.	**The Effect of Macrophage M2 Polarization on Ebola Virus Infection**
	Kai Rogers, Ayithan Natarajan, Wendy Maury Department of Microbiology, University of Iowa, Iowa City, IA
	Ebola virus (EBOV), a negative sense RNA virus and member of the *Filoviridae* family, is capable of causing tremendous morbidity and mortality. It is appreciated that cells of the innate immune system are important target populations in the initial stages and dissemination of EBOV infection. Our findings indicate the activation status of macrophages significantly impacts the susceptibility of these cells to EBOV infection. Ex vivo, M-CSF-matured resident peritoneal macrophages from C57BL/6 interferon α/β receptor (IFNAR) knockout mice were treated with well-established activation cytokines to elicit an anti-inflammatory M2a phenotype, which was confirmed by qRT-PCR for expression of appropriate marker genes. Cells were infected with a BSL-2 model virus (EBOV/rVSV) expressing GFP and infection was quantified at 24 hours by flow cytometry. We demonstrate that macrophages generated by IL4/IL13 treatment were significantly more susceptible to EBOV/rVSV infection than M-CSF-treated cells. We found that IL4/IL13-treated macrophages bound more EBOV/rVSV than M-CSF-treated cells, suggesting that the enhancement of infection of these cells may be due to enhanced receptor expression. To explore this, expression of C-type lectins and phosphatidylserine receptors that might mediate EBOV entry were assessed. We found RNA levels of SIGNR3 (a murine ortholog of human DC-SIGN) to be significantly higher in the IL4/13 treated macrophages than in M-CSF treated cells. Competitive inhibition studies using mannan reduced EBOV/rVSV entry in IL4/13-stimulated macrophages and ectopic SIGNR3 expression enhanced EBOV/rVSV infection, consistent with a role for SIGNR3-dependent uptake in M2a macrophages. In vivo studies demonstrated that mice exposed to IL4/IL13 24 hours prior to infection showed greatly elevated morbidity and mortality compared to PBS-treated littermates. These results demonstrate that M2a polarization enhances infection at least in part by elevating expression of the C-type lectin, SIGNR3. Further, this is the first demonstration that murine SIGNR3 serves as a receptor for EBOV.

4.	**The MHV packaging signal promotes virulence and prevents a robust Interferon response during infection.**
	Jeremiah Athmer, Anthony Fehr, Mathew Grunewal, Stanley Perlman Department of Microbiology, University of Iowa, Iowa City Iowa, 52242
	During Coronavirus replication, two species of viral RNA are replicated, genomic RNA (gRNA) and sub-genomic RNA (sgRNA). These RNAs are co-terminal on both their 5' and 3' ends, containing the leader sequence and 3' UTR/ polyA respectively. Even with these similarities, Coronaviruses are adept at selectively packaging gRNA over sgRNA. This selective packaging is determined, at least in part, by RNA secondary structures present within the coding regions of the non-structural proteins. For lineage A beta-Coronaviruses, a 95 base pair stem-loop structure, termed the Packaging Signal (P/S), is present within the coding region of non-structural protein 15. This RNA motif is sufficient for packaging of nonviral RNAs and has been shown to interact with the M protein from MHV. Moreover, when this RNA motif is altered in MHV, (MHV_{PS}) selective packaging is lost during infection as sgRNAs become a larger percentage of packaged viral RNA. This deletion does not affect viral replication but decreases fitness when placed in competition with wild-type MHV. Following these observations, we assessed the effect of selective packaging on pathogenicity *in vivo.* We found that wild-type mice infected with MHV_{PS} had significantly better outcomes compared to MHV wild-type infected mice. Furthermore, we found peak viral loads were similar between MHV-JHM_{WT} and MHV-$JHM_{P/S}$. Importantly, we found that MHV_{PS}-infected bone marrow derived macrophages had significant increases in type-I interferons (IFNs) and pathogenesis of MHV_{PS} was restored in mice deficient in IFN signaling. These data indicate that the P/S of MHV is an uncharacterized MHV virulence factor, which acts by preventing a robust IFN response during infection.

5.	**The 3' Untranslated Region of Maize Chlorotic Mottle Virus Contains a New Type of Cap-independent Translation Enhancer**
	Elizabeth J. Carino[1,2], W. Allen Miller[1,2], Kay Scheets[3], Keisuke Komoda[1], Sung Ki Cho[1,4], Jelena Kraft[1] [1]Department of Plant Pathology & Microbiology, [2]Interdepartmental Genetics & Genomics, Iowa State University, Ames, IA; [3]Dept. of Plant Biology, Ecology, and Evolution, Oklahoma State University, Stillwater, OK; [4]*Current address:* Biochemistry & Cellular and Molecular Biology Department, University of Tennessee, Knoxville, TN
	Maize chlorotic mottle virus (MCMV) is the key player in the synergistic interaction with potyviruses that causes maize lethal necrosis disease (MLND). An expanding MLND epidemic is causing serious yield losses across East Africa. As a member of the *Tombusviridae* family, MCMV lacks the 5' cap required for conventional translation of host mRNAs. A series of deletions in the viral genome revealed the presence of a 3' cap-independent translation element (CITE) in the 3' untranslated region (UTR), as is the case for other tombusvirids. The MCMV 3'-CITE (MTE) was mapped between nucleotides 4164-4333 (168 nt) in the viral genome, however, the minimal core sequence needed for translation stimulation of uncapped viral or luciferase reporter mRNA in wheat germ and in oat protoplasts was narrowed to nts 4201-4300. SHAPE probing of the core MTE displayed a hammerhead-shaped structure, roughly similar to the previously characterized panicum mosaic virus class 3'-CITE (PTE), but with longer stems, larger terminal loops, and it lacks key conserved bases required for PTE function. As for PTEs, EMSA assays indicated that the MTE binds translation initiation factor 4E (eIF4E). Similar to other 3'-CITEs, long-distance base pairing is predicted between a loop in the MTE and a loop in the 5'-UTR, and mutations that disrupt this interaction reduced MTE activity. Ongoing research focuses on the role of the MTE in MCMV replication, and on identifying mutations in eIF4E that disrupt binding to the MTE. Such mutations may provide novel resistance strategies against this deadly virus.

6.	**Insights into Epstein-Barr virus biology through the study of RNA structure**
	Walter Moss
	Epstein-Barr virus is a ubiquitous human herpes virus that infects > 95% of the adult population. Latent infection with EBV is associated with a number of cancers and serious autoimmune disorders; however, the exact mechanism of how EBV contributes to disease remains unclear. One understudied aspect of EBV biology has been the role of viral RNA structure in the progression/maintenance of infection and its contributions to viral pathogenesis. To begin the process of unraveling the roles played by RNA structure, a comprehensive genome-wide map of structural propensity has been generated for EBV using bioinformatics and comparative sequence analysis. This was then combined with the results from small RNA sequencing to identify novel EBV noncoding RNAs and potential regulatory structures throughout the viral transcriptome. These results provide leads for future studies to understand how these highly-structured elements regulate EBV biology and mediate host-virus interactions relevant to disease.

7.	**Long-distance interactions in the xrRNA structure are required for exonucleolytic generation of the 3' noncoding subgenomic RNA of barley yellow dwarf virus**
	Keisuke Komoda, W. Allen Miller Plant Pathology & Microbiology Department, Iowa State University
	Viruses in the *Flaviviridae* (e.g. dengue virus) and *Tombusviridae* (e.g. red clover necrotic mosaic virus, RCNMV) families generate noncoding subgenomic RNAs (ncsgRNA, called SR1f for RCNMV and sfRNA for flaviviruses) corresponding to the 3' untranslated region (UTR). These ncsgRNAs may act as sponges to sequester specific host and viral proteins that regulate gene expression and host antiviral defense. These ncsgRNAs are generated by a host exonuclease, which degrades the viral genome 5' to 3' until it reaches an RNA structure called xrRNA that blocks its movement, leaving the 3' UTR intact as ncsgRNA. Barley yellow dwarf virus (BYDV, genus *Luteovirus*, *Luteoviridae* family), generates three 3'-coterminal subgenomic RNAs (sgRNA 1, 2 and 3) in infected cells. sgRNA1 serves as mRNA for four ORFs, sgRNA2 encodes ORF6 and regulates translation *in trans*, while the highly abundant, noncoding sgRNA3 (an ncsgRNA) comprises the 330 nt at the 3' end of the genome. We discovered that sgRNA3, but neither sgRNA1 nor sgRNA2, is generated from genomic RNA by exonucleolytic degradation in plant extracts, and by commercial yeast Xrn1 enzyme. sgRNA1 and sgRNA2 can also serve as exonuclease substrates to generate sgRNA3. Mutagenesis and structural probing by SHAPE revealed that, to block the exonuclease, the BYDV xrRNA domain requires (i) a simple stem-loop with a mismatched pair (DG = -5.5 kcal/mol) at the 5' terminus, (ii) up to 10 bases adjacent 3' to the stem-loop, and (iii) sequence(s) near the 3' end of the genome, upstream of the origin of negative strand synthesis. This differs from xrRNA of flaviviruses and RCNMV, in which the entire xrRNA structure is at the 5' end of the ncsgRNA. Thus, unpredicted, long-distance RNA interactions – a common theme in tombusvirids and luteoviruses - appear to control BYDV ncsgRNA synthesis.

8.	**Identification of a conserved coiled-coil-like motif in retroviral Rev-like proteins**
	Chijioke N Umunnakwe[1, 2], Drena Dobbs [1,3], Karin S. Dorman[1,3,4], and **Susan Carpenter**[2] [1]Program in Bioinformatics and Computational Biology, Departments of [2]Animal Science, [3]Genetics, Developmental and Cell Biology and [3]Statistics, Iowa State University, Ames, IA 50011
	Rev-like proteins are essential post-transcriptional regulatory proteins found in several retrovirus genera, including lentiviruses, betaretroviruses, and deltaretroviruses. These essential proteins mediate the nuclear export of incompletely spliced viral RNA, and act by tethering viral pre-mRNA to the host CRM1 nuclear export machinery. Although all Rev-like proteins are functionally homologous, they share less than 30% sequence identity. To assess the extent of structural homology among retroviral Rev-like proteins we undertook a comprehensive analysis of overall protein domain architecture and predicted secondary structural features for representative members of the Rev-like family of proteins. Similar patterns of a-helical domains were identified for Rev-like proteins within each genus, with the exception of deltaretroviruses, which were devoid of a-helices. Coiled-coil oligomerization motifs were also identified for most Rev-like proteins, with the notable exceptions of HIV-1, the deltaretroviruses, and some small ruminant lentiviruses. In Rev proteins of primate lentiviruses, the presence of predicted coiled-coil motifs segregated within specific primate lineages: HIV-1 descended from SIVs that lacked predicted coiled-coils in Rev; whereas, HIV-2 descended from SIVs that contained predicted coiled-coils in Rev. Phylogenetic ancestral reconstruction of coiled-coils for all Rev-like proteins predicted a single origin for the coiled-coil motif, followed by three losses of the predicted signal. The absence of a coiled-coil signal in HIV-1 was associated with replacement of canonical residues found in coiled coils with non-canonical residues. Despite these changes, the a-helical region of HIV-1 Rev oligomerization domain could be modeled as a helical wheel with two predicted interaction interfaces that mapped to the dimerization and oligomerization interfaces in published HIV-1 Rev crystal structures. Analysis of other retroviral Rev-like proteins, including endogenous sequences, revealed similar interaction interfaces that could mediate oligomerization. These findings suggest suggests that the coiled-coil motif constitutes an ancestral and homologous mechanism of oligomerization in retroviral Rev-like proteins.

9.	**Foxtail Mosaic Virus Based Vectors for Gene Silencing and Gene Expression in Maize**
	Yu Mei, Chunquan Zhang, Bliss M. Kernodle, Mingsheng Qi, Katherine L. Quandt, John H. Hill, and Steven A. Whitham Department of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa 50011 (Y.M., B.M.K., M.Q., K.L.Q., J.H.H., S.A.W.); Department of Agriculture, Alcorn State University, Lorman, Mississippi 39096 (C.Z.); and DuPont Pioneer, Johnston, Iowa 50131 (B.M.K.)
	Plant viruses have been widely used as vectors for foreign gene expression and virus-induced gene silencing (VIGS). Viral vectors capable of expressing heterologous proteins in plants and silencing endogenous plant genes provide valuable biotechnological tools to complement conventional genetic technologies. Maize is the most important row crop grown in the USA, but effective virus-based tools for rapidly silencing or overexpressing genes have not been widely adopted due to the lack of a system that is relatively simple to use, consistent, and causes little damage to the host. The ability of *Foxtail mosaic virus* (FoMV), a monopartite plus-stranded RNA virus, to infect maize and other monocots while causing mild symptoms made it a candidate for viral vector development. We describe here a new DNA-based system derived from FoMV that is able to transiently silence endogenous maize genes or express heterologous proteins in the sweet corn line Golden x Bantam. The applications of this FoMV vector system are demonstrated by silencing *phytoene desaturase* (*pds*, a gene in the carotenoid biosynthesis pathway) and *lesion mimic 22* (*les22*, a gene that encodes a key enzyme of the porphyrin pathway). To demonstrate expression of heterologous proteins, green fluorescent protein (GFP) and bialaphos herbicide resistance (BAR) were expressed. We further demonstrated that the FoMV vector establishes systemic infection in maize inbred lines, sorghum, and green foxtail, indicating that it has potentially broad utility for functional genomics studies in maize and other monocots.

10.	**Pathogenesis and Cross-protection of U.S. PEDVs**
	Jianqiang Zhang, Qi Chen, Joseph Thomas, Phillip Gauger Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011
	Porcine epidemic diarrhea virus (PEDV) is the causative agent of porcine epidemic diarrhea (PED), which was first reported in England in the early 1970s and has since spread to other European and Asian countries. In North America, PEDV was detected for the first time in the U.S. in April 2013 and subsequently detected in Canada and Mexico. So far, at least two genetically different PEDV strains have been identified in the U.S. (U.S. PEDV prototype strain [also called Non-S-INDEL strain] and U.S. PEDV S-INDEL-variant strain). The most striking sequence differences between the U.S. prototype and U.S. variant PEDVs are located in the S1 portion. Herein we summarize some features of pathogenicity and cross-protective immunity of U.S. PEDVs based on work conducted in our laboratory. First, the oral minimum infectious dose (MID) of PEDV in naïve conventional neonatal piglets and weaned pigs were determined. A U.S. virulent PEDV prototype isolate (USA/IN19338/2013) with known infectious titer was serially ten-fold diluted and orogastrically inoculated into 7 groups of 5-day-old neonatal pigs (n=4/group) and 7 groups of 21-day-old weaned pigs (n=6/group). In neonatal pigs, 10ml of inoculum having titers 560-0.056 $TCID_{50}$/ml (Ct 24.2-37.6) caused 100% infection and titer 0.0056 $TCID_{50}$/ml (Ct>45) caused infection in 25% of inoculated pigs. In weaned pigs, 10ml of inoculum with titers 560-5.6 $TCID_{50}$/ml (Ct 24.2-31.4) caused 100% infection while titers 0.56-0.0056 $TCID_{50}$/ml did not establish infection. The data indicated that PEDV infectious dose is age-dependent with a significantly lower MID for neonatal than weaned pigs. Secondly, the virulence/pathogenicity differences of three U.S. PEDV prototype isolates and one S-INDEL-variant isolate were compared in naïve neonatal piglets. Fifty 5-day-old pigs were randomly divided into 5 groups (10 pigs/group) and orogastrically inoculated (10 ml; 1×10^4 $TCID_{50}$/ml) with one of 3 U.S. PEDV prototype isolates (USA/IN19338/2013, USA/NC35140/2013, USA/NC49469/2013), or a U.S. PEDV S-INDEL-variant isolate (USA/IL20697/2014), or virus-free culture medium. Compared to the pigs inoculated with the three U.S. PEDV prototype isolates, the pigs inoculated with the S-INDEL-variant isolate 1) had significantly less severe clinical signs; 2) had significantly less fecal virus shedding; 3) had significantly less severe gross lesions in small intestines, ceca and colons; 4) had significantly less severe histopathological lesions in small intestines; 5) had significantly lower immunohistochemistry (IHC) scores in ileum; 6) had lower but non-significantly-different IHC scores in ceca and colons. However, the U.S. PEDV prototype and the S-INDEL-variant strains induced similar viremia levels in inoculated pigs. Thirdly, serological cross-reactivity and cross-neutralization between the U.S. PEDV prototype and S-INDEL-variant strains were evaluated using experimentally-generated antisera against the two virus strains and 5 serological assays: 1) PEDV IFA antibody assay; 2) virus neutralization test; 3) prototype whole virus-based ELISA; 4) prototype S1-based ELISA; and 5) variant S1-based ELISA. It was found that antibodies against the U.S. PEDV prototype and S-INDEL-variant strains cross-reacted and cross-neutralized the two strains *in vitro*. Fourthly, the cross-protection efficacy between the U.S. PEDV prototype and S-INDEL-variant strains were examined in weaned pigs. Eighty-five PEDV-naïve 3-week-old pigs were randomly divided by weight into 7 groups with 15 or 10 pigs per group. Pigs were orogastrically inoculated with virus-negative culture media, PEDV prototype strain virus isolate USA/IN19338/2013, or PEDV S-INDEL-variant virus isolate USA/IL20697/2014 at Day 0 (D0) followed by challenge at D28. It was found that U.S. prototype PEDV provided protection against challenge with prototype or S-INDEL-variant strains; U.S. S-INDEL-variant PEDV provided protection against S-INDEL-variant strain challenge and at least partial protection against prototype strain challenge in weaned pigs.

11.	**Cellular stress response to varicella-zoster virus infection of human skin includes both elevated interleukin-6 transcription and robust autophagy**
	John E. Carpenter (a), **Erin M. Buckingham** (a), Wallen Jackson (a), Keith W. Jarosinski (b), Kevin Knudtson (c) and Charles Grose (a) Virology Laboratory, Children's Hospital (a), Department of Microbiology (b), Iowa Institute of Human Genetics (c), University of Iowa, Iowa City, IA
	The infectious cycle of varicella-zoster virus (VZV) includes a viremia followed by replication and assembly of complete virions in the human skin to cause the characteristic vesicular rash (exanthem). We have previously documented cellular stress demarcated by CHOP and spliced XBP1 followed by autophagic flux in infected monolayer cultures. To pursue additional studies of innate immunity to VZV infection and crosstalk with autophagy, we postulated that a more accurate representation would include VZV infection of human skin explants. To that end, we adapted a fetal skin-organ-culture (SOC) model to human foreskin SOC. Comparative studies documented that the infected SOC model was an authentic representation of VZV infection of human skin. Samples for RNA extraction were obtained from VZV-infected monolayers and VZV-infected SOC, along with uninfected monolayers, uninfected SOC and Poly(I:C) stimulated cells. Microarray experiments demonstrated differing patterns of upregulated transcription between VZV-infected cells and VZV-infected skin explants. After examination of the results from the 84 wells among different innate immune transcripts, one result stood out, namely, a 32-fold elevated IL-6 level in the infected skin explant that was not present in an infected monolayer culture. IL-6 transcription was elevated to the same high level in cells stimulated with Poly(I:C). Furthermore, both 2D and 3D imaging demonstrated that VZV induced autophagosome formation was more plentiful than ever seen previously in VZV-infected monolayers. Thus, cellular stress in response to VZV infection leads to both elevated IL-6 transcription and robust autophagosome formation.

12.	**Dynamics of semen exosome-mediated HIV inhibition**
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	The AIDS pandemic remains a significant global problem with more than 36 million people living with HIV-1 worldwide. It is estimated that greater than 80% of HIV cases result from sexual transmission, making it the most prevalent mode of transmission, of which semen is the primary vector. However, less than 1% of acts of heterosexual coitus with an infected individual result in HIV infection, suggesting that in addition to the protective effect of the mucosal environment, factors contained in semen may impair the infectivity/fitness of HIV. Previously, we have shown that human semen from HIV-negative men is enriched with exosomes that potently impair HIV-1 infectivity. Here we show that semen exosomes (SE) decrease the levels of all HIV RNA species (multiply spliced; singly spliced; and unspliced) in infected cells, and that the reduction in HIV RNA is conserved in progeny virions released in the presence of SE. Additional data show that SE decrease i) the level of genomic RNA in the cytoplasm, ii) encapsulation efficiency of HIV RNA into progeny virions, and iii) progeny reverse transcriptase activity. Surprisingly, HIV cell- and virion-associated protein levels are unchanged in the presence of SE, indicating that virus production is unaffected by SE. These results do suggest that SE may impair HIV RNA transcription and/or encapsidation.

13.	**Introduction of a fluorescent competent tetracysteine tag within mammalian orthoreovirus non-structural protein μNS for visualization of viral factory dynamics**
	Luke D. Bussiere[1,2], Promisree Choudhury[1], Bryan Bellaire[1,2], Cathy L. Miller[1,2] [1]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University College of Veterinary Medicine; [2]Interdepartmental Microbiology Program, Iowa State University, Ames, IA 50011
	Viral factories (VF) are intracellular, viral inclusions that form during host infection. Mammalian orthoreovirus (MRV) utilizes VFs, organized by non-structural protein μNS, as sites for viral transcription, translation, core assembly and replication. Considerable research has examined VFs to better describe their function as VFs are not well characterized. Currently it is understood that viral protein μNS forms VFs and recruits viral proteins and the core particle to VFs. To study VFs researchers have primarily visualized μNS and associating proteins in fixed cells illustrating a snapshot of VF activity. While live cell microscopy could demonstrate VF dynamics over time a major hurdle in the ability to study VFs in live cells has been the inability to recover recombinant virus expressing fluorescent μNS. Thus we set out to develop a method using a small six amino-acid tetracysteine (TC)-tag, along with FlAsH-EDT2 fluorescing reagent, to tag μNS. We introduced the TC-tag within eight regions of a μNS encoding M3 gene expression plasmid to identify regions of μNS amenable to the TC-tag. We observed TC-μNS formation of viral factory-like (VFL) structures as well as FlAsH-EDT2 labeling, and performed viral protein colocalization studies to determine which TC-μNS mutant behaved most similar to wildtype μNS. Upon examination of eight mutants we identified six that maintained the capacity to form VFL structures which were also competent for FlAsH-EDT2 labeling, of which three maintained their ability to associate with most μNS interacting proteins. We performed reverse genetics to recover recombinant TC-μNS virus and were successful in recovering two recombinant viruses that were subsequently used to measure VF dynamics using live cell microscopy. VFs in infected cells exhibited substantial movement including short locational shifts as well as individual VF fusion and transient interaction events. This work advances our ability to study VF function in live MRV-infected cells.

14.	**Ebolavirus Glycoprotein Residue 95 Alters Endosomal Requirements for Virus Entry**
	Nicholas J. Lennemann[1], **Jacob Dillard[1]**, Ashley Cooney[1], Grace Shaack[1], Robert A. Davey[2], Wendy Maury[1] Department of Microbiology, University of Iowa, 51 Newton Road, Iowa City, IA 52242[1];Department of Virology and Immunology, Texas Biomedical Research Institute, 620 NW Loop 410, San Antonio, TX 78227[2]
	Amino acid polymorphisms found in Ebola virus (EBOV) glycoprotein (GP) can affect the efficiency of virus entry into cells. This is exemplified by the single amino acid change, A82V, recently identified in GP that alters the ability of the Makona strain of EBOV to enter human cells. Here, we investigated a GP polymorphism present in the GP of two of the earliest Sudan virus (SUDV) isolates, where a glutamine is found at residue 95 (Q95) within the receptor-binding domain of GP1. In contrast, in more recent SUDV isolates and all GPs from other ebolaviruses a lysine is present at this position (K95). We found that this polymorphism altered titers of VSV virions pseudotyped with SUDV GP, with Q95 having 20-fold lower titers than K95. In contrast to SUDV GP, introduction of Q95 into EBOV GP had no effect on titers and Q95 in Bundibugyo GP enhanced titers. Thus, the impact of this polymorphism is strain specific. We investigated the effect of the polymorphism on different steps of entry. The presence of Q95 led to greater GP sensitivity to proteolytic processing of all three GP. In contrast, the effect of K95Q on inhibitors of cholesterol trafficking, Ca^{2+} fluxes, and sphingomyelinase activity such as 3.47, verapamil and imipramine were strain-specific, although entry of all six GPs required NPC1 interactions. These findings suggest that late events in entry are influenced by residue 95, but the effects of that residue are influenced by additional strain-specific GP residues. Our findings highlight a potential problem with development of entry antivirals that are efficacious against all filoviruses since the presence of this single natural polymorphism results in profound and unpredictable changes in virion sensitivity to numerous entry inhibitors. Further, this serves a cautionary note that entry inhibitors need to be evaluated against the breadth of *Ebolavirus* species.

15.	**Extragenic Suppression of a Mutation in Herpes Simplex Virus Type-1 (HSV-1) that Affects Lamina Disruption and Nuclear Egress**
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	Localized disruption of the nuclear lamina is considered an essential step in the replication of HSV-1. Isolation and characterization of viral mutants that affect lamina disruption can be used as a powerful tool to study the dynamics of lamina assembly and disassembly. Viral nucleocapsids are assembled in the nucleus and escape into the cytoplasm via an envelopment process termed nuclear egress. The nuclear lamina is thought to act as a steric barrier to nuclear egress: viral capsids are too large and the meshwork of the lamina is too rigid to accommodate the interpenetration of viral capsids. Viral proteins pUL31 and pUL34 create the nuclear egress complex (NEC), which is required for multiple steps of the nuclear egress pathway, including nuclear lamina disruption. In current models of nuclear lamina disruption, localized dissociation occurs through phosphorylation of lamin subunits and lamin-associated proteins (LAPs). HSV-1 mediated lamina disruption results in a change in nuclear shape and the distribution of lamin proteins. We have characterized a pUL34 mutant protein (UL34Q163A) that inhibits lamina disruption and nuclear egress. The pUL34 mutant localizes properly to the nuclear periphery and interacts with pUL31, but results in a 100-fold reduction in plaque size and a 50-fold reduction in virus production. Nuclear morphometry and immunofluorescence detection of lamin protein distribution shows that this mutant is defective in disrupting the nuclear lamina. Upon selection of extragenic suppressors of UL34Q163A, a mutation in pUL31 (UL31R229L) was found to partially compensate the virus production and plaque size defect of pUL34Q163A, but it did not recover the ability to disrupt the lamina. Our findings suggest that lamina disruption is not an essential step for nuclear egress of HSV-1.

16.	**Interaction of HIV-1 Env with Membrane Lipids Maintains the Trimer in a Closed Functional Conformation**
	Jacklyn Johnson, Hamid Salimi, Michael Zhang, Manuel Flores, Yunxia O'Malley, Jon Houtman, Patrick Schlievert and Hillel Haim Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA
	The fusion proteins of many enveloped viruses are contained in plasma membrane domains that have a unique lipid and protein composition. Agents that deplete cholesterol from virion membranes efficiently inactivate their infectivity. Exposure of viruses to surfactants that incorporate into the lipid membrane can also inactivate viruses. However, the mechanisms underlying inactivation by both types of agents are still unclear. We examined the effects of cholesterol depletion and surfactant microbicides on structure of the membrane-bound envelope glycoproteins (Envs) of HIV-1. Chemical depletion of cholesterol induces an 'open' conformation of Env, increasing exposure of cryptic epitopes that overlap the coreceptor- and CD4-binding sites. Similar conformational changes are induced by mutations in the putative cholesterol-interacting motif of Env. The surfactant microbicides glycerol monolaurate (GML) and nonoxynol-9 (N-9) induce structural changes similar to disruption of the Env-cholesterol interaction. Interestingly, deletion of the cytoplasmic tail (CT) of Env increases virus resistance to inactivation by cholesterol-depleting and surfactant microbicides. Therefore, interactions between Env and the viral membrane maintain trimers in a 'closed' functional conformation. Deletion of the CT decreases the dependence of Env on such structure-stabilizing interactions. Our findings suggest that different microbicides that alter the lipid composition of the membrane inactivate HIV-1 by a common mechanism. These concepts may apply to the fusion proteins of other enveloped viruses, which demonstrate similar patterns of sensitivity to inactivation by agents that perturb the lipid membrane.

17.	**Equine influenza elicits multiple broadly neutralizing antibodies to hemagglutinin**
	Sarah A. Wiechert[1], Jessie Trujillo[6], Richard Webby[4], Sandhya Bangaru[5], James Crowe[5], Brian Lee[3], Brett A. Sponseller[1,2], and David Verhoeven[3] Departments of Veterinary Microbiology and Preventive Medicine[1], Veterinary Clinical Sciences[2], Biomedical Sciences[3], Iowa State University; St. Jude Children's Research Hospital[4]; Vanderbilt Vaccine Center[5];Veterinary Diagnostics Laboratory, Kansas State University[6]
	Influenza continues to pose a serious threat to public health, especially for humans and swine. Influenza strains are continuously circulating within and between populations and species creating significant viral shift and drift. Current influenza vaccines have demonstrated limited efficacy in humans (0-68%) and only limit or prevent morbidity and mortality in swine, not infection. Universal vaccines have proven challenging to design due to antigenic variability, difficulty in targeting the more conserved stalk, or vaccine-enhanced respiratory disease in swine. We have recently discovered that vaccinating two different mammalian species (mice and horses) with equine H3N8 (live attenuated) led to the broadest neutralizing antibody profile to date (within strains: H1N1, H3N2 including recent swine cross-over H3N2v2, and H5N1 with further binding to H7, H9, and H13 HAs) and protected from multiple H1N1 and H3N2 influenza challenges. If this vaccine should work in swine or humans, it could represent a significant advancement toward more efficacious influenza vaccines.

18.	**Antiviral strategies against human respiratory syncytial virus (RSV) in perinatal and newborn lambs**
	Mark R. Ackermann Department of Veterinary Pathology, Iowa State University, 2738 Veterinary Medicine, Ames, IA 50011-3619
	Respiratory syncytial virus (RSV) is common cause of respiratory disease and a leading cause of bronchiolitis and hospitalization in infants. Due to tragic deaths related to experimental formalin-inactivated vaccines in the 1960's, there are no fully effective vaccines. Although Palivizumab (Synagis, MedImmune) has protective properties therapeutically and Ribavirin has been used in some clinical situations, there are also no fully satisfactory therapeutic regimens. Thus, there is a need to develop new therapeutic approaches in a relevant animal model. Newborn and preterm lambs have developmental, structural, cellular, physiologic, and immunologic features similar to infants and also can be infected with human strains of RSV with lesions similar/identical to those of infants, including bronchiolitis. These features and lesions are now well characterized and we have used newborn lambs to test various therapeutic strategies including: delivery of vascular endothelial cell growth factor (VEGF), activation of the Dual function oxidase (Duox)/lactoperoxidase system with potassium iodide, nebulization of anti-RSV camelid nanobody, and delivery of RSV fusion inhibitors. In addition, we have demonstrated that preterm lambs have reduced innate and adaptive immune responses that pre-dispose lambs to increased RSV disease severity and that maternal ethanol consumption during gestation reduces a) pre- and term lamb lung endogenous hyopoxia-inducible factor (HIF) and endogenous VEGF levels and b) surfactant protein A (SP-A) mRNA levels. Thus, the newborn lamb model of RSV infection is conducive to studies of therapeutic intervention; especially those that are mechanistic and at a fundamental level.

19.	**Determination of the immunodominant regions of Senecavirus A-VP1 by ELISA epitope mapping**
	Houston ER[1], Gimenez-Lirola LG[1], Sinha A, Shen H, Villarino NF[2], Piñeyro PE[1] [1]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA. [2] Pharmacology/Individualized Medicine program, Washington State University, College of Veterinary Medicine, Pullman Washington
	Senecavirus A (SVA) is a RNA virus of the genus *Senecavirus* in the family *Picornaviridae.* The virus was recently detected in swine production in the U.S., causing sporadic vesicular lesions and neonate mortality. The structural VP1 protein has proven highly immunogenic in SVA and other *Picornaviridae* members. However, no information on immunodominant regions of SVA-VP1 is available. The objective of this study is to identify immunodominant regions of SVA-VP1 using ELISA epitope mapping. Experimental anti-SVA polyclonal antibodies (SVA-pAb) and SVA-VP1 monoclonal antibodies (VP1-mAb) were characterized by whole virus (WV) and recombinant VP1-ELISA (rVP1-ELISA), WV and VP1 western blot, virus neutralization and immunofluorescence assays. The complete SVA-VP1 sequence was truncated into 18 overlapping peptides shifted by 5 amino acids. All peptides were evaluated against SVA-pAbs and VP1-mAbs by indirect ELISA. All experiments were controlled using pre-injection mouse sera. A peptide blocking ELISA was used to assess the blocking effect of each peptide on SVA-mAb binding activity to rVP1 immobilized to the surface of polystyrene microplate. The half maximal inhibitory concentration (IC50) was calculated for each peptide comparing 100% binding activity of SVA-mAb to SVA-rVP1. No significant differences in reactivity against WV or rVP1 was observed between SVA-pAbs and VP1-mAbs evaluated by WV and rVP1-ELISA and Western blot, and IFA. However, viral neutralizing was detected for SVA-pAbs but not for VP1-mAbs. All VP1-derived peptides were reactive against SVA-pAb and VP1-mAb, with no significant difference in reactivity amongst peptides except for peptide-1 by indirect ELISA. Four epitopes showed a potent inhibition in binding activity between rVP1 and Vp1-mAb. These results suggest that immune response generated by SVA-VP1 in mice can be defined by a set of linear epitopes that may be involved in the neutralizing activity of antibody. Further investigations warranted to determine potential neutralizing activity induced by this set of linear epitopes.

20.	**Nanovaccine-mediated immune protection against influenza A virus**
	Zeb R. Zacharias[a], Kathleen A. Ross[c], Jonathan T. Goodman[c], Balaji Narasimhan[c], Thomas J. Waldschmidt[a], and Kevin L. Legge[a,b] [a]Immunology Graduate Program and Department of Pathology, [b]Department of Microbiology, University of Iowa, Iowa City, IA 52242 [c]Department of Chemical and Biological Engineering, Iowa State University, Ames, IA, 50011
	Influenza A virus (IAV) is a major cause of serious respiratory illness. Worldwide, IAV infections lead to approximately 5 million severe cases and 500,000 deaths per year. This significant morbidity and mortality, along with the associated economic burden and the discontinued use of live-attenuated influenza vaccination (i.e. FluMist®), collectively stress the need to develop novel and efficacious IAV vaccination strategies. Recent studies indicate immunizations that generate tissue-resident memory T and B cells at the local site of the infection (i.e. nasal mucosa and lung) provide the best protection against future IAV encounters. However, currently licensed IAV vaccines primarily induce systemic IAV-specific antibody responses and do not induce lung-resident memory populations. Thus, our objective is to develop an IAV vaccine platform with the capacity to induce lung-resident T and B cells. Herein, we test the efficacy of a biodegradable polyanhydride nanoparticle-based IAV vaccine (IAV-nanovax) to produce lung-resident, cross-protective humoral- and cellular-mediated immune responses. Our results demonstrate mice that received an i.n. IAV-nanovax inoculation were protected against subsequent homologous and heterologous IAV challenges. These mice exhibited reduced viral loads within the lungs during such challenges and possessed lung-resident IAV-specific $CD4^+$ and $CD8^+$ T cells. Furthermore, these mice had lung-resident germinal center B cell responses and produced IAV-specific IgG and IgA responses. These results strengthen the concept that polyanhydride nanoparticles hold the capacity to induce tissue-resident immune responses that protect against homologous and heterologous IAV infections.

Poster

Presentations

P1.	**The role of NLRP12 during influenza A virus infection**
	Emma E. L. Hornick[1], Kevin L. Legge[1], Gail A. Bishop[1,2], Suzanne L. Cassel[3], Fayyaz S. Sutterwala[1,3] [1]Interdisciplinary Graduate Program in Immunology, University of Iowa, Iowa City, IA; [2]VA Medical Center, Iowa City, IA; [3]Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA
	Members of the nucleotide-binding domain and leucine-rich-repeat-containing (NLR) family of pattern recognition molecules participate in host immune responses to a wide variety of pathogens. We recently reported that mice lacking NLR family member NLRP12 have an increased susceptibility to bacterial infections that correlates with impaired neutrophil migration. Given the importance of neutrophils for survival of high-dose influenza A virus (IAV) infection, we sought to determine the role of NLRP12 in this context. Surprisingly, NLRP12-deficient mice exhibited significantly less morbidity and mortality compared to wild-type (WT) mice following infection with a lethal dose of IAV, despite having fewer pulmonary neutrophils than WT mice at early and late time points post-infection. Quantification of neutrophil chemoattractants CXCL1, CXCL2 and CXCL5 revealed that only CXCL1 was decreased in the lungs of NLRP12-deficient mice. Consistently, NLRP12-deficient bone marrow-derived dendritic cells (BMDC) produced less CXCL1 than WT BMDC following IAV infection *in vitro*. Together, these data support a previously unappreciated role for NLRP12 in exacerbating pathogenesis of IAV infection.

P2.	**Divergent macrophage induced lung pathology occurs between infants/toddlers and adults during respiratory viral infection**
	David Verhoeven Department of Biomedical Sciences, College of Veterinary Medicine, 2008 Vet Med, Ames, IA, 50011
	Young children, especially those aged 6 months to 2 years of age, frequently exhibit severe morbidity during respiratory viral infections. For influenza infections, macrophages/monocytes serve as front line defenses against early viral replication in the lungs until the adaptive immune system arrives to clear virus. However, infiltrating inflammatory monocytes are a significant cause of influenza induced lung pathology and morbidity. We recently developed a young murine model of respiratory viral infections using 21-day-old mice to investigate the mechanisms driving the heightened influenza induced morbidity observed in human young children. We hypothesized that macrophages/monocytes responses to influenza would diverge between young and older mice despite evidence that macrophages from both groups appear to control viral replication at similar rates. While inflammatory monocyte infiltration contributed to influenza induced morbidity/lung inflammation in adults, they did not appear to contribute to morbidity in young mice. Instead, young mice appeared to develop lung inflammation through local proliferation of alveolar macrophages with limited monocyte recruitment. While intrinsic limitations in anti-viral cytokine responses, especially interferon gamma, characterized the macrophage response to viral infection in young mice, they were still able to control viral replication by clustering near sites of active viral infection with concentration of their limited anti-viral cytokines over infected epithelial cells, especially near the bronchioles. This study highlights the intrinsic limitations in macrophage effector functions that may arise in young children but that also contribute to disease pathology.

P3.	**Virucidal effects of MST-312 and Epigallocatechin gallate on *Herpes Simplex Virus 1***
	Prajakta Pradhan and Marie L. Nguyen Dept. of Microbiology & Immunology, Des Moines University, Des Moines, IA 50312
	Herpes Simplex Virus (HSV) is the cause of cold sores, blindness and brain damage and often leads to recurrent infections. The current anti-viral therapies can fail when drug resistant HSV mutants arise. Thus, novel drugs for the treatment of HSV are needed. Previous research in our laboratory has determined that the telomerase inhibitor, MST-312, interferes with multiple steps of the HSV life cycle. The structure of MST-312 contains moieties related to a natural compound found in green tea, epigallocatechin gallate (EGCG). EGCG has been reported to possess direct virucidal activities toward HSV-1. Here, we test the virucidal activity of MST-312 and compare it to that of EGCG. Specifically, HSV-1 was exposed to various concentrations of MST-312 or EGCG for time periods between 1 and 60 minutes and then the ability of the treated virions to form plaques on Vero cells was assessed. When treated for 30-60 minutes, 40 μM MST-312 and 0.5-1.0 μM EGCG significantly reduced the number of HSV-1 plaque forming units. The temperature at which treatment occurred impacted the ability of the compounds to limit viral replication. Both compounds were effective when treatment occurred at 37^{o}C and room temperature (RT). However, no inhibition was seen when virions were treated with MST-312 at 4^{o}C. One minute of treatment with 2 μM EGCG at RT was sufficient to significantly reduce HSV titers. These data indicate that both EGCG and MST-312 possess direct virucidal properties on HSV-1. Higher concentrations of MST-312 were required to inactivate HSV-1 virions compared to EGCG.

P4.	**Identification and Characterization of Small Molecule Inhibitors of Porcine Reproductive and Respiratory Syndrome Virus**
	Alyssa B. Evans[a], Pengfei Dong[b], **Hyelee Loyd[a]**, George A. Kraus[b], Susan Carpenter[a] Department of Animal Science, Iowa State University, Ames, IA, 50011[a]; Department of Chemistry, Iowa State University, Ames, IA, 50011[b]
	Porcine reproductive and respiratory syndrome virus (PRRSV) is poorly controlled by the currently available vaccines. Because of this, outbreaks occur and often spread quickly within and between both vaccinated and unvaccinated herds. Alternative control strategies are needed to help prevent the continual circulation of the virus. Antivirals have proven to be an extremely successful treatment strategy for several human viral diseases, and research efforts have recently begun to identify antivirals for use in treatment of livestock diseases. Previously, the natural compound atractylodinol was reported to have anti-PRRSV activity *in vitro*. In the current study, we synthesized atractylodinol and thirteen analogs to characterize their anti-PRRSV activity *in vitro*. Six of the analogs had potent inhibitory activity against 5-$\log_{10}$ infectious units of PRRSV at 390 nM to 2.2 μM concentrations. Analog compound 9 was shown to inhibit PRRSV primarily at a post-attachment step during PRRSV entry. These results provide evidence that the atractylodinol analogs are promising antiviral candidates for trials in pigs.

P5.	**Virus discovery and applications for the management of snail-vectored human disease**
	Sijun Liu, Si-Ming Zhang, Eric S. Loker and Bryony C. Bonning, Department of Entomology, Iowa State University, USA; Department of Biology, The University of New Mexico, USA
	A World Health Organization resolution targets the elimination of schistosomiasis as a public health concern by the year 2025. Taking advantage of next generation sequencing (NGS) technologies and our efficient bioinformatics pipeline developed for identification and confirmation of virus-derived sequences from NGS data, we identified virus-derived sequences from the transcriptomes of three snail vectors of schistosomiasis: *Biomphalaria glabrata, Biomphalaria pfeifferi* and *Bulinus globosus*. Sequences derived from more than 20 novel viruses were identified from 27 RNA sequence datasets, with 8 near full-length small RNA virus genomes assembled. All viral sequences showed similarity on BLAST analysis to picorna-like viruses of diatoms or unknown marine viruses. More than seven of the snail-derived virus sequences showed similarity to the dicistrovirids and iflavirids of insects, along with *Maranvirus*, *Bacillarnavirus* and *Labyrnavirus* isolated from aquatic environments. Phylogenetic analysis based on RNA-dependent RNA polymerase or coat protein sequences revealed that some virus sequences were likely derived from ingested aquatic unicellular organisms. Other virus sequences that clustered close to, but separate from insect viruses in the phylogenetic tree are hypothesized to be snail viruses. The potential practical application of such viruses for snail management will be discussed.

P6.	**Putative receptor for Israeli acute paralysis virus of the honey bee, *Apis mellifera***
	Shunji Li, Amy L. Toth and Bryony C. Bonning Department of Entomology, EEOB, Iowa State University, Ames, IA 50011, USA; Present address: Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611
	International concern over ongoing colony losses of the honey bee, *Apis mellifera*, has resulted in increased knowledge of the multiple factors that negatively impact honey bee health. Viruses of the honey bee, including Israeli acute paralysis virus (IAPV), appear to play a major role in colony declines. IAPV has a positive sense, single stranded RNA genome and belongs to the family Dicistroviridae. Infection occurs via ingestion of virions, which are predicted to be taken up into gut epithelial cells via receptor-mediated endocytosis. Here, we report a putative midgut receptor for IAPV. The putative receptor was identified by far-western blotting. Brush border membrane vesicles (BBMV) were prepared from dissected honey bee midgut tissues and proteins separated and analyzed by 2-dimensional ligand blot. IAPV virions were used as ligand with detection mediated by IAPV anti-VP1 antiserum. Peptide sequences of candidate receptor proteins were determined by LC-MS/MS, with proteins identified with reference to the translated genome of the honey bee. The identification of a putative receptor for IAPV can provide valuable information for future strategies to mitigate or prevent virus infection and associated mortality in honey bee colonies.

P7.	**BST-2/Tetherin potentiates motility of virus infected cells: Potential for virus dissemination**
	Wasifa Naushad, Wadie D. Mahauad-Fernandez, Chioma M. Okeoma Department of Microbiology and Immunology, University of Iowa, USA, Iowa City, IA 52242
	The restriction factor BST-2/tetherin plays a critical role in preventing the release of progeny virions from infected cells and in promoting viral clearance through activation of signal transduction pathways. Although BST-2-mediated effect limits cell-free virus infection and spread, what happens to the infected cells ladened with unreleased virions is yet to be determined. We hypothesize that BST-2-tethered viruses may alter the molecular signature of infected cells thereby regulating cell-to-cell viral spread. Using acute retrovirus infection model and various cell lines (cervical epithelia-derived TZM-bl, breast-epithelia-derived 4T1 cells, monocytic U937, and SUP T cells), we show that the titer of extracellular virus significantly increases while intracellular viral load decreases in BST-2-suppressed cells compared to their BST-2-expressing counterparts. Despite equivalent starting cell numbers, BST-2-expressing cells exhibit increased proteolytic and non-proteolytic cell motility. The motile cells contain high levels of viral nucleic acids, BST-2, and metalloproteases. Interestingly, increased cell motility of BST-2-expressing cells correlates with increased infection of target cells in a trans-infection model of virus transmission. We conclude that BST-2 may promote virus spread by enhancing the ability of virus-ladened cells to migrate, carve through extracellular membranes, and infect a population of target cells at distal sites.

P8.	**In vitro replication of PRRSV in MDM established from genetically susceptible and resistant pigs**
	Hyelee Loyd, Alyssa Evans, **Miranda Kapfer**, Jack C M Dekkers, Susan Carpenter Department of Animal Science, Iowa State University
	Porcine reproductive and respiratory syndrome (PRRS) caused by infection with the same-named virus (PRRSV) is a globally important disease causing significant economic losses in the swine industry worldwide. A region of high LD on swine chromosome 4 (SSC4) was found to explain genetic variance associated with viral production and weight gain of PRRSV-infected pigs. Pigs with the undesirable A allele had increased in viral production and decreased weight as compared to pigs with the desirable, dominant B allele. Possible candidate genes from this SSC4 region are several IFN-stimulated genes, including members of the guanylate binding protein (GBP) family. GBP1 and GBP5 have been shown to have direct antiviral activity *in vitro*, and a polymorphism in GBP5 results in alternative splicing and premature truncation in AA, but not AB, pigs. Together, these findings raise the possibility that differences in PRRSV replication in vivo occurs at the cellular level. To examine this, we analyzed PRRSV replication in monocyte-derived macrophages (MDM) established from AA, BB and AB pigs. Cells were infected at MOI 0.001 and supernatant was collected at sequential times post-infection. Viral production and infectivity were analyzed using quantitative RT-PCR and titration on MARC-145 cells, respectively. There were no significant differences in virus production, virus infectivity, or the kinetics of virus replication associated with pig genotype. This suggests that genetic control of PRRSV replication by SSC4 results from complex interactions among immune cells in vivo.

P9.	**The HIV-1 Env Trimer Adopts a Unique Conformation in Seminal Plasma and Semen Simulants**
	Manuel Flores[1], Jacklyn Johnson[1], Marlon Mendez[1] and Hillel Haim[1]. [1] Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA
	The envelope glycoproteins of HIV-1 mediate entry of the virus into host cells. Binding of high-affinity ligands (e.g., cell-surface receptors or inhibitory antibodies) can alter structure of the Env trimer. Small changes in temperature can also affect exposure of different Env epitopes. Interestingly, the effect of the medium in which viruses are contained *in vivo* and transmitted structure of Env is less well characterized. We examined the effect of seminal plasma and a semen simulant solution on structure of the Env trimer. To define structural properties we measured binding of probes to Envs expressed on HOS cells using a cell-based ELISA system. We found that relative to standard tissue culture media, the Env trimer assumes a more open conformation in semen. Binding of trimer-dependent probes is reduced whereas exposure of the coreceptor-binding site is increased. In addition, engagement of glycan-targeting antibodies to the Env trimer is reduced. The unique conformational arrangement of Env trimers in semen may affect the potency of vaccine-elicited antibodies to prevent sexual transmission at mucosa sites.

P10.	**TiPARP Upregulation During CoV Infection Reveals Potential Activation of the Aryl Hydrocarbon Receptor**
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	ADP-ribosylation is a post-translational modification that plays a role in cellular DNA replication repair, cellular stress response, protein degradation, and inflammation. ADP-ribose can be covalently attached to proteins as mono-ADP-ribose (MAR) or poly-ADP-ribose (PAR) by ADP-ribosyl transferases (ARTDs or PARPs). The role of ADP-ribosylation in virus replication and pathogenesis has only been minimally addressed. Coronaviruses (CoVs) are a family of positive-sense RNA viruses with human and agricultural significance, and all CoVs encode a macrodomain in non-structural protein 3 (Nsp3) that can remove ADP-ribose from target proteins. Importantly, the mouse hepatitis virus (MHV) macrodomain mutant virus (N1347A) replicated poorly and induced an enhanced interferon response in bone marrow-derived macrophages (BMDMs). This indicates that ADP-ribosylation likely plays a role in CoV replication or pathogenesis. To identify the PARP(s) that may modulate infection, we analyzed the expression of PARP family members in BMDMs. We identified a small subset of PARPs that were significantly expressed and upregulated following infection with MHV. Furthermore, upregulation of at least one PARP, TiPARP, was maintained in interferon alpha/beta receptor-defective macrophages, suggesting that IFN-independent mechanisms contribute to its expression. TiPARP is known to be activated by the aryl hydrocarbon receptor (AHR), a transcription factor that responds to tryptophan metabolites and regulates the immune response. We then tested if other factors upstream and downstream of AHR are also upregulated during MHV infection. Our results show that AHR and genes upstream and downstream of AHR are significantly upregulated during infection. In conclusion, we have identified a small number of PARPs that may play a role during CoV infection. Furthermore, our preliminary results suggest that MHV induces AHR activation which could play a role in the immune response to virus infection.

P11.	**Type I interferons and alveolar macrophages protect hDPP4-KI mice from mouse-adapted strain of MERS-CoV infection.**
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	Middle East Respiratory syndrome (MERS) is a lethal pulmonary infection caused by a newly identified coronavirus (CoV), MERS-CoV. Since its emergence in 2012, MERS-CoV infected 1888 individuals with 670 deaths. However, due to a lack autopsy samples and relevant mouse models, pathogenesis of MERS is poorly understood. We recently developed a mouse model with a humanized locus for the MERS-CoV receptor, dipeptidyl peptidase 4 (DPP4) (hDPP4 knock-In mice). Infection of hDPP4 KI mice with mouse-adapted MERS-CoV (MERS-CoV$_{MA}$) caused lethal pneumonia and replicated many of the features of MERS in humans. Although protective during multiple acute virus infections, IFN-I response is either protective (MHV-1) or pathogenic (SARS-CoV) following coronavirus infection. Consequently, using hDPP4 KI mice, we investigated the role of IFN-I following MERS-CoV challenge. Infection of hDPP4 KI mice with a sub lethal dose (300 PFU) MERS-CoV$_{MA}$ resulted in peak virus replication and IFN-I response around day 2 p.i. In contrast to infection with SARS-CoV, blocking IFN-I signaling resulted in increased morbidity and mortality in hDPP4 KI mice after MERS-CoV$_{MA}$ infection. High mortality following abrogation of IFNAR signaling was associated with enhanced virus replication, altered pro-inflammatory cytokine/chemokine responses and lung immunopathology characterized by inflammatory cell accumulation and alveolar edema. Blocking IFN-I signaling also impaired virus-specific T cell responses in the lungs. Additionally, depletion of alveolar macrophages increased the susceptibility of hDPP4 Ki mice to MERS-CoV$_{MA}$ infection mimicking the effect of blocking IFN-I signaling, suggesting that macrophages could be a potential source of IFN-I during MERS-CoV infection. Together, our results demonstrate that IFN-I signaling and alveolar macrophages play a critical role in host protection during MERS-CoV infection.

P12.	**Adaptive characteristics of NK cells in anti-influenza virus immune response**
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	NK cells represent for one of major components of innate immunity and play crucial roles in anti-viral and anti-tumor immune responses. However, their roles during secondary immune responses against infectious pathogens are still exclusive. In this study, by using mouse adaptive influenza virus PR8 and multiple recombinant viruses, we demonstrated some adaptive characteristics of NK cells during influenza virus infections.

P13.	**The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Exhibits Bilateral Entry and Exclusively Apical Egress in Well-Differentiated Human Airway Epithelial Cells**
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	Middle East Respiratory Syndrome coronavirus (MERS-CoV) is a zoonotic virus that can cause high mortality in human (~35%). The clinical spectrum of MERS-CoV infection may range from asymptomatic or mild respiratory symptoms to severe acute respiratory disease and death. The human airway epithelium (HAE) cells is the primary site of infection for respiratory viruses. To assess the target cells and consequences of viral infection in the airways, we studied MERS-CoV infection in primary air-liquid interface cultures of well-differentiated human airway epithelial (HAE) cells derived from tracheobronchial tissues. We found the expression of virus receptor (dipeptidyl peptidase 4 (DPP4)) which correlated to MERS-CoV infection is highly variable from different donors. In well-differentiated human airway epithelia, MERS-CoV can infect cells from either the apical or basolateral surface in a DPP4-dependent fashion. However, the entry pathway may be different because apical infection is sensitive to protease inhibitors while basolateral infection is not. Regardless of the polarity of entry, virus release was exclusively from the apical surface. The initial infection occurred predominantly in non-ciliated cells, but some ciliated cells also can be infected. Throughout the course of the infection, infected epithelial cells were cleared from airway by sloughing and this procedure may accelerate viral clearance. There is no evidence of loss of integrity of the epithelial barrier. Apical release favors MERS-CoV spread by respiratory droplets.

P14.	**Upregulation of Proinflammatory Cytokines following Infection with Senecavirus A in NCI-H1299 cells**
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	Senecavirus A (SVA) has oncolytic properties and is the causative agent of idiopathic vesicular disease in pigs. The objective of this study was to characterize the transcription and expression profile of proinflammatory cytokines (i.e., IL-6, IL-12p35, IL-12p40 and TNF-α) in the human non-small cell lung carcinoma cell line NCI-H1299 infected with SVA. NCI-H1299 cells were divided into 4 infection groups treated with 0.0001, 0.001, 0.01 and 0.1 multiplicity of infection (MOI) of SVA, one control treated with 1 μg/ml of lipopolysaccharide (LPS) from E.coli, and one negative control (NC) treated with normal medium. Samples for determination of proinflammatory cytokine gene expression and production, virus titration, and cell cytotoxicity were collected at 6, 12, 24 and 36 hours post infection (hpi). Obvious cytopathic effect (CPE) was observed in 0.01 MOI group at 36 hpi, and in 0.1 MOI group at 24 and 36 hpi. No obvious CPE was observed in 0.0001 MOI, 0.001 MOI, LPS and NC groups. The intensity of luminescence detected by Viral ToxGlo Assay (Promega) indicated that the 0.1 MOI group had the lowest cell viability at 36 hpi. The titration of the cell culture supernatant revealed that SVA successfully propagated in NCI-H1299 cells in all infection groups, with higher titers in 0.1 and 0.01 MOI groups than that in 0.001 MOI and 0.0001 MOI groups. The relative quantification of proinflammatory gene transcription in NCI-H1299 cells by real-time RT PCR revealed a significant upregulation of hIL-6, hIL-12p35, hIL-12p40 and hTNF-α mRNA at 36 hpi in 0.1 MOI group. Cytokine protein level detected by a Luminex method revealed that 0.1 MOI group had the highest Median Fluorescent Intensity on hIL-6 and hTNF-α at 36 hpi. Our data suggest that infection of NCI-H1299 cells with SVA increases the transcription of proinflammatory cytokines in a dose dependent manner.

P15.	**Measles virus mediated reorganization of the actin cytoskeleton in primary human airway epithelia**
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	Measles virus (MeV) is a highly contagious respiratory virus that continues to be a worldwide health burden. MeV infection of immortalized cells results in syncytia formation, whereas in well-differentiated primary cultures of airway epithelial cells from human donors (HAE) MeV forms infectious centers without visible syncytia formation, and without compromising epithelial function. We have shown that once infection is established, MeV spreads rapidly from cell-to-cell via intracellular pores (J. Virol. 89, 7089-96, 2015). We recently observed actin cytoskeletal reorganization within epithelial cells at the center of infectious centers but not in cells along the periphery. Here we investigate the role of the actin cytoskeleton in the process of cell-to-cell spread in HAE. HAE were treated with cytoskeleton disrupting or stabilizing drugs and both the number and size of infectious centers were quantified from 1 to 3 days post-infection. Of note, in cells treated with the small molecule inhibitor of formin homology 2 (SMIFH2), which disrupts formin-dependent but not Arp2/3 complex dependent actin cytoskeletal structures, a significant reduction in infectious center size was observed. Furthermore, using super resolution microscopy, we showed that the MeV phosphoprotein and nucleocapsid protein extensively co-localize with actin filaments at the apical side of HAE. Our results suggest that MeV spreads rapidly in airway epithelial cells by assembling on the cytoskeleton and travelling between cells along the cytoskeletal adhesive belt.

P16.	**Assessment of ELISA discrepant Equine Infectious Anemia (EIA) samples submitted to the National Veterinary Services Laboratories**
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	In 2015, there were approximately 1.3 million equine infectious anemia (EIA) tests performed in the United States. The majority of these tests are routine, for transport or surveillance purposes. A small percentage test positive on any one of the four commercially available enzyme-linked immunosorbent assays (ELISA). A discrepant sample is forwarded to the National Veterinary Services Laboratories (NVSL) for confirmatory testing. A discrepant sample typically includes a positive or equivocal result on ELISA but negative on any other test. An equivocal result is a result that is questionable or ambiguous. NVSL tests the sample by all commercially available ELISAs and by agar gel immunodiffusion (AGID). The NVSL algorithm indicates that when a sample is positive or equivocal on two or more ELISAs and negative on AGID, a Western blot (WB) is performed. For calendar year 2015, NVSL received 252 discrepant samples that resulted in a negative EIA status of the horse following confirmatory testing. Of those, 22 were positive on ELISA kit "A", 35 were positive with 3 equivocal on ELISA kit "B", 102 were positive with 6 equivocal on ELISA kit "C", and 33 were positive with 2 equivocal on ELISA kit "D". Fifty-six of the 252 samples tested positive on two or more ELISAs. For these 56 samples, WB was performed. Ninety-three of the 252 samples were negative on all tests performed. While ELISA tests are highly sensitive, they are not specific enough to eliminate false positive reactions observed during routine EIA testing, thus requiring additional testing to verify the EIA status of the animal. Testing of each sample by all commercially available tests allows identification of potential performance issues with the test kits.

P17.	**Evaluation of a novel multi-immunogen vaccine strategy for targeting 4E10/10E8 neutralizing epitopes on HIV-1 gp41 membrane proximal external region**
	Saikat Banerjee[a,†], Heliang Shi[a,†], **Marisa Banasik**[a,†], Hojin Moon[a,§], William Lees[b,§], Yali Qin[a], Andrew Harley [a], Adrian Shepherd[b] and Michael W. Cho[a] [a]Department of Biomedical Sciences, Iowa State University, Ames, IA 50011; [b]Institute of Structural and Molecular Biology, Birkbeck College, University of London, UK
	The membrane proximal external region (MPER) of HIV-1 gp41 is targeted by broadly neutralizing antibodies (bnAbs) 4E10 and 10E8. In this proof-of-concept study, we evaluated a novel multi-immunogen vaccine strategy referred to as Incremental, Phased Antigenic Stimulation for Rapid Antibody Maturation (IPAS-RAM) to induce 4E10/10E8-like bnAbs. Rabbits were immunized sequentially, but in a phased manner, with three immunogens that are progressively more native (gp41-28x3, gp41-54CT, and rVV-gp160$_{DH12}$). Although nAbs were not induced, epitope-mapping analyses indicated that IPAS-RAM vaccination was better able to target antibodies towards the 4E10/10E8 epitopes than homologous prime-boost immunization using gp41-28x3 alone. MPER-specific rabbit monoclonal antibodies were generated, including 9F6. Although it lacked neutralizing activity, the target epitope profile of 9F6 closely resembled those of 4E10 and 10E8 (^{671}NWFDITNWLWYIK^{683}). B-cell repertoire analyses suggested the importance of co-immunizations for maturation of 9F6, which warrants further evaluation of our IPAS-RAM vaccine strategy using an improved priming immunogen.

P18.	**West Nile Virus Infection in Human and Mouse Cornea Tissue**
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	The purpose of this study was to determine the *in vitro* and *ex vivo* susceptibility of human corneal cells to West Nile virus (WNV) infection and evaluate the ability of the virus to disseminate to the corneas of infected mice. Human corneal epithelial cells were challenged with WNV, incubated for 1 to 6 days, and tested for evidence of WNV infection. Viral RNA and antigen were detected at every time point and the virus reached a peak titer of 2.5×10^7 plaque-forming units per milliliter (pfu/ml) at 3 days post-inoculation (PI). Corneas procured from donors were incubated in culture dishes containing WNV for 1 to 5 days and tested for evidence of WNV. Viral RNA and antigen were detected and the virus reached a mean peak titer of 4.9×10^4 pfu/ml at 5 days PI. Mice were inoculated intraperitoneally with WNV, and their eyes harvested at 2, 5, and 8 days PI and tested for evidence of WNV. Viral RNA was detected in anterior segment tissues in 4 of 9 systemically infected mice as early as 2 days PI. We conclude that human corneal cells support WNV replication *in vitro* and *ex vivo*, and WNV may disseminate into the corneas of experimentally infected mice. These findings indicate that corneal transmission cannot be ruled out as a novel mode of human-to-human WNV transmission and additional experiments should be conducted to assess this risk further.