American Association of Veterinary Immunologists

Message from AAVI President Chris Davies

After a two-year apprenticeship under Past Presidents Lorraine Sordillo and Joan Lunney I am honored to take over as the President of the American Association of Veterinary Immunologists (AAVI). Over the past couple of years our association's membership has increased and we have been much more successful with fund raising. Consequently, the AAVI is now on sound financial footing. Joan Lunney, our Past President, has been a great mentor and it is my pleasure to thank Joan for her enthusiasm, dedication and great leadership. I would also like to thank Eileen Thacker, the AAVI Secretary-Treasurer for the past three years, for her long-standing commitment to the AAVI. Eileen has helped to position the AAVI for the future by updating our membership database and website, and by her efforts to strengthen our financial situation. In addition, I want to thank Ron Shultz, the Chair, and the other members of the Fundraising Committee for their efforts to bring in corporate support for our association. Without corporate support we would be hard pressed to carry out many of the activities of our association. If anyone has contacts at companies that might be interested in becoming corporate (supporting) members of the AAVI, I would encourage you to contact Ron Shultz or one of the other members of the Fundraising Committee.

A complete list of AAVI Officers and Committee Members is included later in the newsletter. I welcome Gina Pighetti, Secretary-Treasurer, Doug Bannerman, Vice President, Krishna Murthy, Board Member, David Hurley and Randy Sacco, Nomination Committee Members,

Incoming AAVI President Chris Davies presents Joan Lunney a commemorative plaque for her service as President in 2006-2007.

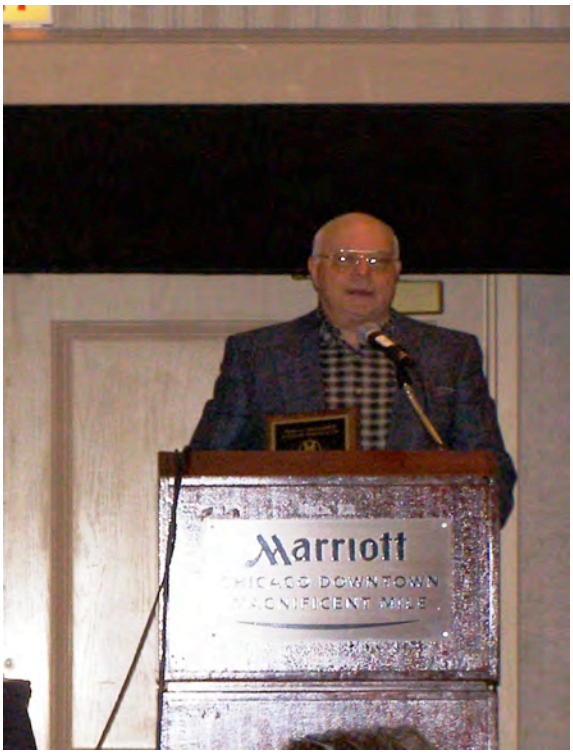
and Susan Eicher, our Newsletter Editor to their new positions. I also thank all of the Officers and Committee Members for their contributions to the AAVI.

A couple of important decisions that were made at our recent board meeting were to make subscriptions to *Veterinary Immunology and Immunopathology* optional for a additional fee, rather than using 50% of your membership fees to provide online journal access to all AAVI members, and to re-implement the student membership category. Hopefully, these changes will help ensure the financial stability of the AAVI and attract new members. Gina Pighetti has also been working on implementing an online credit card payment system that will make it easier for members to pay their

dues on an annual basis. We should have this available by the time the newsletter comes out or shortly thereafter. I would also like to encourage members to contribute to the Jeanne Burton Memorial Fund. Jeanne, who died this past August following a courageous battle with colon cancer, was an active member of AAVI and a great mentor of graduate students and post-doctoral fellows. Following Jeanne's family's wishes, the AAVI will use these funds to honor Jeanne's memory by sponsoring student awards in her name. Donations can be made either by sending a check to Gina Pighetti, the AAVI Secretary-Treasurer, or by using the online donation form, which will be available shortly as a link from the AAVI website.

An important activity of the AAVI is to facilitate and encourage the participation of young veterinary immunologists at scientific meetings by providing travel stipends and awards for outstanding presentations. The AAVI provided seven Junior Scientist Travel Stipends for the 8th International Veterinary Immunology Symposium (IVIS), which was held in Ouro Preto, Brazil in August 2007. These travel stipends where supported by a $10,000 grant from USDA, CSREES. The abstracts from the IVIS travel stipend awardees were included in our last newsletter. An annual activity for the AAVI is to support student awards for presentations in veterinary immunology at the Conference of Research Workers in Animal Diseases (CRWAD). Extended abstracts from the first and second place awardees in the oral and poster competitions at the December 2007 CRWAD are presented later in this newsletter. I congratulate the IVIS travel stipend and CRWAD award recipients on their excellent work. It is always impressive to see the accomplishments of young members of the veterinary immunology community. I also thank Carol Chitko-McKown, the chair of the AAVI Student Awards Committee, and the judges for their dedication to the student awards competition. Finally, I would like to again congratulate Dr. John Butler from the University of Iowa on being chosen as both the AAVI and IUIS-VIC Distinguished Veterinary Immunologist for 2007. Dr. Butler's studies on the comparative immunology of immunoglobulins have certainly been seminal studies in immunoglobulin biology.

One of the core missions of the AAVI is to promote veterinary immunology in the broader scientific community. In her January 2001 AAVI Presidential Message Cynthia Baldwin presented several definitions of veterinary immunology. One definition that she presented was the one used by the journal *Veterinary Immunology and Immunopathology*, which is "studies of any aspect of immunology conducted in agricultural and companion animals as well as wildlife." For most of us, our primary objective is to understand the immunological mechanisms involved in control of infectious diseases, immunopathology or immunodeficiency in veterinary species, rather than to do comparative of phylogenetic studies. Nevertheless, one of the ways to interest human and rodent immunologists in studies in veterinary immunology is to highlight the comparative aspects of how the immune system functions in different species. For many human diseases a veterinary species may be a better model than rodents. Although it is important to attend veterinary meetings, such as IVIS and CRWAD, I would encourage you to attend smaller specialized immunology meetings that focus on specific aspects of immunology and to present your work with



Dr. John Butler is 2007 Distinguished Veterinary Immunologist.

veterinary species. For the past eight years I have been active in the American Society for Reproductive Immunology. This has broadened my understanding of comparative immunology, has promoted recognition of my research from outside of the veterinary immunology community, and has made it possible for me to gain funding for my research from NIH. Consequently, I would encourage all of our members to reach out to the broader scientific community.

Thanks to our outgoing AAVI Secretary Treasurer, Eileen Thacker

This year marks an important transition for the AAVI as the office of Secretary Treasurer moves from Eileen Thacker at Iowa State University to Gina Pighetti at the University of Tennessee. Eileen worked diligently to upgrade the AAVI's electronic capacity.

She improved our AAVI website http://www.theaavi.org/ with the help of her colleague, Chris Minion at Iowa State University She watched over our funds and invested them well.

Eileen Thacker tackled many tasks to improve the function of AAVI before resigning in 2007.

She took on the onerous task of registering AAVI on grants.gov so that we could apply for USDA CSREES meeting travel grants. She successfully piloted the grant that funded the travel of 7 graduate students and postdoctoral fellows to present their research at the 8th International Veterinary Immunology Symposium in Ouro Preto, Brazil.

Eileen's efforts, along with those of Carol Chitko-McKown, Chair of AAVI's Student Awards Committee, have highlighted the accomplishments of our future veterinary immunologists. They organized and supported our many student based initiatives and awards.

With Elsevier management, Eileen worked to assure that AAVI members received electronic access to Veterinary Immunology and Immunopathology as part of their membership.

We all give our thanks to Eileen for her efforts for the AAVI. We also wish her well as she transitions into her new position as a National Program Leader for Animal Health at the USDA ARS in Beltsville MD.

Incoming Secretary Treasurer's Report,
By Gina Pighetti

Hello everyone!

Thank you for the vote of confidence in selecting me as the next Secretary/Treasurer of AAVI. For those who do not know me, I am a faculty member in the Department of Animal Science at the University of Tennessee. Although on a steep learning curve at the moment, I welcome all comments and suggestions you all may have to improve our organization. I also want to thank Eileen once again for her service to AAVI and for her help and future patience while we are in the process of transferring her duties to me.

In keeping with Eileen's moving us forward in the digital world, we are in the process of giving our members the option to pay by credit card through Google Checkout. To use this service we are developing web links that will be accessible on our membership page. Simply click on the links and it will connect you to the Google Checkout site. If you have already used this service for other purchases, you can simply login and make your payment. Otherwise, you will need to register with Google Checkout prior to making payment. An added bonus is that most of our international members will be able to use this service as well.

We are in the process of updating the website over the next month, so please stay tuned to

http://theaavi.org for this opportunity to pay by credit card. If you would like to know more about Google Checkout and its security please visit Google's website.

In honor of our colleague, AAVI has established the ***Jeanne Burton Memorial Fund.*** Jeanne was an Associate Professor in Animal Science at Michigan State University and was best known for her work in immunity using immunogenomics approaches to understand periparturient immunosuppression and mastitis susceptibility in dairy cows. Jeanne was a strong advocate and mentor of graduate students and post-doctoral fellows – including myself. Her drive and passion will be missed greatly. Following Jeanne's family's wishes, the AAVI will use these funds to honor Jeanne's memory by sponsoring student awards in her name. Please consider giving a donation.

Your membership dues help us organize and support various veterinary immunology programs at meetings such as CRWAD, AAI, and IVIS, as well as support scholarship and travel awards for students. So, please send in your dues. To maintain active status, dues should be paid by March 1, but I will gladly take your money anytime. We also re-instated a reduced rate for students of $20 so please encourage your students to join. I will continue to take checks made payable to AAVI and sent to me at the University of Tennessee. As mentioned above, in the very near future you also will be able to pay dues, VII fees, and make donations to the Jeanne Burton Memorial Fund through Google Checkout.

Gina Pighetti answers questions about the Google payment system.

If you haven't been receiving e-mails from AAVI it may be that your contact information is out of date. Please check the membership PDF file to see if we have your current contact information. If not, please send me an e-mail with your updated info so we can keep you up-to-date. In closing, if you have questions regarding membership, any suggestions for improving how we do things or potential new options please feel free to touch base with me (pighetti@utk.edu).

Fund Raising Committee Report
By Ron Schultz, Chair

Dear AAVI Members,

As chair of the Fund Raising Committee, I would like every member to help AAVI raise funds to sponsor our many activities. The following are a few of the fund raising activities where we can use your help:

1) Pay your dues
2) Recruit more full time and student members
3) Identify individuals in industry that we can contact about "Corporate Membership in AAVI"

We have several companies that are long time members, a few companies that occasionally pay dues or provide a gift, and others that have never given any money to AAVI. If you are employed by a company, try to get your company to join AAVI. Annual dues are $1,500. If the company doesn't want to be a member or pay the $1,500, ask them to donate whatever amount to AAVI to help with our "travel awards" or to sponsor one of our symposia. Members who have good friends or contacts in companies that may have an interest in AAVI, talk to them about membership or about making a gift to AAVI. If you don't want to ask your company or friend in a company about membership or a gift, please let me or other members of the Fund Raising Committee know and we will be pleased to ask your company or friend in a company for money.

Thank you for all of your help.

Ronald D. Schultz, Professor and Chair
Department of Pathobiological Sciences
School of Veterinary Medicine
University of Wisconsin-Madison
2015 Linden Drive
Madison, WI 53706-1102
Tel. (608) 263 9888
schultzr@svm.vetmed.wisc.edu

Nominations Committee Report: Nominations Needed for Key AAVI Positions

By Jim Roth, Chair

AAVI Members are invited to submit nominations (or to volunteer themselves) for several key leadership positions in AAVI and for the 2008 Distinguished Veterinary Immunologist (DVI). This year, nominations are needed for: 1) Vice President, who will serve for a four year period, moving from Vice President to President Elect, to President and finally Past President; 2) Board Member, also with a four year term; and 3) two Nominations Committee members, (three year term). The Nominations Committee will accept nominations, perhaps make additional nominations, and select candidates for the annual ballot. We invite you to participate by submitting names of individuals who have been AAVI members for at least three years and are in permanent career positions. Please submit these names to the following e-mail address before May 15th: jaroth@iastate.edu. The committee will then contact the candidates to determine whether they will agree to have their name placed on the ballot. Each year the AAVI Board selects an individual for the Distinguished Veterinary Immunologist (DVI) award. It is the Nomination Committee's responsibility to present to the board, three names of individuals who they believe meet the eligibility requirements and best reflect the intent of the award. The candidate must be an immunologist who is not currently a member of the AAVI Nominations Committee and whose contributions to veterinary immunology are widely acknowledged as significant and important to the understanding of the immunology of domestic and/or wild animals. The award recognizes all specialties of veterinary immunology and is open to members and non-members of AAVI from any country. The Nominations Committee will select three candidates. The membership is encouraged to submit the names of individuals meeting these criteria. However, in this case we ask that the name be submitted formally through a letter of nomination sent to the address below indicating why the individual should be considered and providing evidence for that belief. The letter must be received on or before April 15th. On behalf of the entire Nominations Committee I thank you for your involvement in this selection process.

Jim Roth (2008 Chair)
AAVI Nominations Committee
Director, Center for Food Security & Public Health
College of Veterinary Medicine
Iowa State University
Ames, Iowa 50011

Veterinary Immunology and Immunopathology

By Cynthia Baldwin, Co-Editor-in-Chief

An important update to VII is the addition of a new format for Technical Reports. This allows description of new gene sequences (genomic or expressed), as well as new mAbs, bioactive proteins etc. This format requires peer review of sequences and annotations, etc. and gets them into a print format which GenBank does not. It allows publication of mAb and proteins without requiring application of the information in a research study. Here are the instructions: *A Technical Report* is a description of: (A) a new gene or its expressed sequence (mRNA) that is comprehensive at least with regard to the coding sequence or the complete mature expressed protein, with annotation (leader/signal sequence, start, stops, and other features), comparison to other species (e.g. by cladogram, percent similarity of gene and deduced amino acid), evidence of deposition into a publicly available gene bank (e.g. GenBank with accession number); or (B) a new monoclonal antibody that convincingly shows specificity for a new target molecule or allows significant improvement of existing procedures or diagnostics (ELISA, flow cytometry, Western blotting, tissue sections); or (C) availability of a functional recombinant cytokine or chemokine with clear evidence that it has biological activity commensurate with the native molecule. Headings such as Introduction, Methods etc. are not required. This decision was made at the 8th IVIS in Brazil in August 2007 by the Editorial Board.

In addition, we are always looking for people who will act as Guest Editor to put together a Special Issue on their topic of interest or from meetings and for people to write mini-reviews. We have a new Reviews Editor, Dirk Werling at the

Royal Veterinary College, London, who is actively soliciting these manuscripts.

AAVI/ACVM 2007 Symposium was Successful Despite the Weather

By Chris Chase, Co-Chair

The annual AAVI-ACVM Symposium was held at CRWAD on Sunday, December 2, 2007 at the Chicago Downtown Marriott. The theme of this year's symposium was Nutritional Immunology. The presenters were Joe Urban, USDA/ARS, Beltsville MD, "Probing the role of micronutrients in immunity to parasitic infection in mouse and swine disease models"; Simin Meydani, Tufts University "Nutrition, immune response & infectious diseases in the aged"; Kirk Klasing, University of California, Davis "General mechanisms by which nutrition impacts immunity and resistance to infectious diseases." and Margherita Cantorna, Penn State University "Vitamin D, Immunoregulation and the risk of autoimmunity". Attendance was lower than expected due to winter storms that caused travel delays for many CRWAD participants, including two of the speakers who did not arrive until Sunday.

I would like to thank Chris Davies for pinch-hitting for Jim Harp as co-moderator as Jim could not make the meeting because of the weather. Additionally, Jim Harp needs to be thanked for his outstanding work at getting the program planned and the participants contacted. The meeting was a success due to Jim's due diligence.

For future CRWAD meetings, please plan to arrive early so that you can participate in the AAVI/ACVM symposium. The symposia are an excellent opportunity to get an in depth review of a hot topic in veterinary immunology.

U. S. Veterinary Immune Reagent Network

By Cynthia Baldwin, Project Director

Updates of the recent work by the U.S. Veterinary Immune Reagent Network (US-VIRN) are available on the website: http://www.umass.edu/vetimm/. Please visit the US-VIRN website to see the progress. The US-VIRN Gene Clone Inventory is included at the end this newsletter.

2007 AAVI Student Presentation Competition at CRWAD

By Carol Chitko-McKown, Chair

The 2007 AAVI Student Presentation Competition was held during the 88th annual CRWAD meeting, December 2-4, in Chicago, Illinois. Eighteen students participated in the oral and poster categories, with equal numbers competing within the two categories. The presenters represented 13 universities from the United States and Canada. Research topics spanned the length and breadth of veterinary immunology with over 7 different species being studied including, cattle, horses, cats, chickens, mice and various wildlife species. This alone should prove that our organization is not biased in favor of any one or two model animal species! Three judges were assigned to each of the presentation categories, and the presentations earning the highest overall scores captured the awards. The judges for 2007 were Drs. Scott McVey, Susan Eicher, Carol Wyatt, Laura Miller, Jishu Shi, and Doug Bannerman, and they were a pleasure to work with.

First place in the oral category was awarded to Amanda Adams from the Maxwell H. Gluck Equine Research Center, Department of Veterinary Science at the University of Kentucky, Lexington and second place was awarded to Ravi Kulkarni from the Department of Pathology, Ontario Veterinary College at the University of Guelph, Ontario. The award for best poster presentation went to Lalit Beura from the Nebraska Center for Virology and the Department of Veterinary and Biomedical Sciences at the University of Nebraska-Lincoln. Second place was awarded to Alexandra Elliott from the Department of Animal Science at the University of Tennessee, Knoxville. The AAVI Awards Committee would like to congratulate all of the winners and their mentors, and thank all of the participants. It is always a pleasure to see the enthusiasm our young scientist's have for their chosen research areas!

Finally, we had a very good response to our request that competitors sign up for the Immunology section when submitting their abstracts. PLEASE do so again for the 2008 competition – it makes the judges' input more

equivalent and fair. See you in 2008! Following are extended abstracts of the winning presentations:

1st Place Oral Presentation

Characterization of the immunological and physiological response of aged horses to equine influenza infection

A. A. Adams*, C. C. Breathnach, T. Sturgill, T. Chambers & D. W. Horohov.

Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY.

Equine influenza virus (EIV) is a leading cause of acute respiratory disease in horses. The mortality of adult horses due to EIV infection ranges from 1-20%, while increasing to 60-90% in foals and immunocompromised horses. While old horses (>20 yrs) are classified as immunocompetent due to age-associated changes in immune function, the susceptibility of old horses to EIV infection remains unknown. Nevertheless, influenza is the sixth leading cause of death among the elderly (>65 yrs). Determining the susceptibility of old horses to EIV is important because old horses make up 10-15 % or more of the U.S. equine population and many remain active within the equine industry. Furthermore, there is increased international and domestic travel of horses and increased equine influenza outbreaks worldwide. Thus, characterizing age-associated changes in the immune response to EIV of the old horse is crucial. The effect of age on the immune response to influenza is well documented in the elderly, including decreased seroconversion, seroprotection, cytotoxic T lymphocyte activity, lymphocyte proliferation and interferon-gamma (IFN-γ) production. Yet, the only documented response to influenza in the old horse is decreased antibody production to an inactivated influenza vaccine. Thus, the objective of this study was to determine the physiological and immunological response of aged horses to EIV infection.

We hypothesize the susceptibility to influenza infection to be similar for young (6-12 months) and old (>20 yrs) horses. Eight old horses with previous, but unknown history of exposure to EIV, and six naïve yearlings were challenged with EIV via a 45-minute aerosol exposure in a chamber stall. Clinical signs (coughing, nasal discharge, dyspnea, depression, anorexia), rectal temperature and viral shedding were determined for a period of 2 weeks post challenge. Peripheral blood mononuclear cells (PBMC) were collected and stimulated *in vitro* with influenza prior to and 2 weeks post challenge to determine EIV-induced IFN-γ synthesis (dual staining for $CD5^+$ cell surface antigen and IFN-γ intracellular staining using flow cytometry) and EIV-specific proliferation (stimulation index (SI) using thymidine incorporation). Hemagglutination inhibition (HI) titers were also determined prior to challenge and at 1 and 2 weeks post. Whole blood was collected for 7 days post challenge into Paxgene tubes, RNA isolated, RT into cDNA and RT-PCR performed to determine pro-inflammatory cytokine mRNA production *in vivo*.

Physiological results showed no significant difference in clinical signs between young and old horses post challenge ($P>0.05$). Though, there was a significant difference in the febrile response between young and old horses following the challenge ($P<0.05$). Old horses had a lower baseline temperature compared to young (99.2ºF ± 0.411, 100.3ºF ± 0.440, respectively). Despite the differences, there was a significant increase in the febrile response following EIV infection in both young (day 2, 103.6ºF ± 0.440) and old horses (day 3, 100.8ºF ± 0.411) ($P<0.05$). Likewise, both young and old horses shed virus post infection. However, 62.5% of the old horses shed virus for an average of 1.75 days compared to 100% of naïve yearlings shedding virus for an average of 3.83 days ($P<0.05$) post challenge. There was a significant difference in EIV-induced IFN-γ synthesis between young naive and old horses prior to and post challenge ($P<0.05$). Further, there was a significant increase in the EIV-induced IFN- γ production prior to and post challenge only in the old horse group (pre, 2.26 ± 0.781 & post, 5.03 ± 0.781) ($P<0.05$). While, there was no significant difference between young and old horses in EIV-specific proliferation ($P>0.05$), both groups had an increased proliferation response 2 weeks post challenge. Old horses had significantly higher (log10) HI titers pre and 1, 2 week post challenge compared to young ($P<0.001$). However,

both young and old horses had a significant increase in (log10) HI titers prior to verses post challenge (young pre, 0.690 ± 0.127 and post, 2.55 ± 0.127 & old pre, 1.34 ± 0.110 and post, 2.92 ± 0.110) ($P<0.05$). Both young and old horses exhibited similar increases in IL-1β, IL-6, IFN-γ and IL-10 mRNA post challenge ($P>0.05$). Further, there was a significant increase in IL-1α and IL-6 mRNA production on day 2 post challenge in both young and old horses ($P<0.05$).

In summary, the clinical data show that old horses are just as susceptible to EIV infection as young naïve yearlings, though there were differences in rectal temperature and viral shedding. The differences in EIV-induced IFN-γ production and HI titers likely reflect memory responses due to prior exposure of old horses to EIV. This study provided evidence that old horses are capable of responding immunologically to EIV infection. Therefore, an EIV vaccination protocol for old horses is important. The immune response of old horses to a traditional inactivated vaccine has been shown to be poor. This study indicates that old horses may respond better to a live-agent vaccination protocol and thus remains to be determined.

2nd Place Oral Presentation

Immunization of broiler chickens against *Clostridium perfringens*-induced Necrotic Enteritis and identification of B-cell epitopes in protective antigens

Kulkarni, R. R.*, Parreira, V.R., Sharif, S. and Prescott, J. F.

Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Necrotic enteritis (NE) is an important disease of broiler chickens caused by *Clostridium perfringens*, a Gram-positive anaerobic bacterium. The disease is usually controlled by use of antimicrobials in feed or water. There is increasing interest in reducing antibiotic use because of drug resistance issues. Vaccination offers an alternative prophylactic measure. However, currently no effective vaccine against NE is available and immunity to NE is not well characterized. Our previous study identified certain immunogenic secreted proteins unique to virulent *C. perfringens* that reacted strongly to serum and intestinal antibodies collected from previously infection-immunized immune birds. These proteins, identified by Mass Spectrometry, included alpha-toxin, glyceraldehyde 3-phosphate dehydrogenase (GPD), pyruvate: ferredoxin oxidoreductase (PFOR), fructose 1,6-biphosphate aldolase (FBA), and a Hypothetical Protein (HP).

The current study investigated the role of these proteins in conferring protection to broiler chickens against different severities of oral infection challenge with virulent *C. perfringens*. Genes encoding these proteins were cloned into an expression vector, pET28a and gene products were purified as histidine-tagged recombinant proteins from *E. coli* and used in immunization. Commercial broiler chickens were immunized intramuscularly three times (days 7, 14 and 21) at a dose of 20 µg /bird /injection. Quil-A (50 µg /bird /injection) was used as an adjuvant. Alpha-toxin was immunized in three forms; a toxoided form, the active form and a combination of toxoid/ toxin. The combination consisted of priming with alpha-toxoid and boosting with active toxin. Birds were challenged on day 28 with two different severities (Mild, Severe) of challenge. Mild challenge consisted of feeding of birds with feed mixed with virulent *C. perfringens* given twice a day for 3 days, whereas for severe challenge the duration of feeding was extended to 5 days. The severity of challenge was further confirmed based on the mean lesion scores of the small intestine at necropsy examination. Serum was collected from birds at three times during the experiment (pre-immunization, mid-experiment and pre-challenge) and intestinal washings were collected at the time of necropsy for evaluating antibody responses by ELISA.

All proteins significantly protected broiler chickens against mild challenge. In addition, immunization with alpha-toxin/toxoid, HP and PFOR also showed significant protection against more severe challenge. Neither alpha-toxoid, nor active toxin when used alone, protected birds. This suggests the importance of conformational epitopes of alpha-toxin in protection against NE which is

likely to be subtle to achieve in immunization. However, two other proteins (HP and PFOR) are potential vaccine candidates, reported for the first time in the current study. Antibody responses in immunized birds as measured by ELISA showed significantly higher IgY titres in serum compared to controls. Intestinal IgY responses were higher than IgA in immunized birds compared to controls which may be due to the route of immunization that may have primed systemic immune component more efficiently.

Further, to identify continuous linear B-cell epitopes in HP and PFOR, individual peptides were synthesized (SPOTs Synthesis, Sigma Genosys Biotechnologies, Woodlands, TX) and applied to a derivatized cellulose membrane. The synthesis of peptides using the SPOTs technique was based on the primary sequence of HP and PFOR. Each peptide of 12 amino acids in length and offset by 6 residues synthesized were reacted with polyclonal serum collected from protected birds immunized with HP/ PFOR. Goat anti-chicken IgY (H+L) were used as secondary antibodies. The bound antibodies were detected using the chemiluminescent substrate CDP-Star and enhancer Nitro Block-II and the membrane was visualized under a Molecular Light Imager. The quantified signal of each spot was obtained using Win Light software and the value was expressed as relative percentage of signal intensity. Three regions in the sequence of HP consisting of 4-7 continuous linear peptide epitopes were identified that showed strong affinity for antibodies. The first region contained the predicted active site (GVAHELGHNF- Zinc binding signature motif). In PFOR, four linear epitopes were identified and the fourth peptide contained an active site of this enzyme (AYVCPHAT- Iron binding region).

In conclusion, this study showed for the first time the importance of certain secreted proteins other than alpha-toxin in immunity to NE with potential use as vaccine candidates, and identified important B-cell epitopes in two protective immunogens.

1st Place Poster Presentation

Certain PRRSV proteins inhibit IFN-β promoter activation

L. K. Beura*, B. Kwon, K. Saira, S. Subramaniam, A. K. Pattnaik, C. Jones and F. A. Osorio. Nebraska

Center for Virology and Dept. of Veterinary Biomedical Sciences, University of Nebraska, Lincoln, NE

Type I Interferons (IFNs) are key molecules in innate immune response against viral infection. They stimulate synthesis of many IFN stimulated genes (ISGs), which help in establishing an antiviral state in cells and prevent viral spread. To antagonize anti-viral effects of IFNs, viruses have developed specific antagonists as counter measures. Viral proteins that target type I IFN system either inhibit the production of IFNs or interfere with the downstream signaling pathway elicited by IFNs. Porcine reproductive and respiratory syndrome virus (PRRSV) is a major cause of economic loss to the swine industry. PRRSV has been shown to be a poor inducer of IFN-α. Pigs infected with PRRSV (European type) have no detectable level of IFN-α in broncho-alveolar lavage fluid and relatively low level of IFN-α in serum. *In vitro* PRRSV-infected porcine alveolar macrophages (PAMs) as well as peripheral blood mononuclear cells (PBMCs) exhibit no detectable levels of IFN-α in supernatants. Moreover, even Transmissible gastroenteritis virus (TGEV, a well known inducer of type I IFN) superinfection of PRRSV-infected PAMs could not elicit IFN-α production. North American PRRSV strains were found to be sensitive to recombinant IFN-α treatment and were poor inducer of IFN-α *in vitro*. It has long been speculated that some viral protein(s) is/are mediating this type I IFN antagonistic function. Earlier reports predicted this inhibition to be at the level of IFN-β transcription. To investigate this matter in detail, we screened different viral proteins for their possible role in inhibition of activation of IFN-β promoter. All the viral proteins were cloned individually in a mammalian expression vector. Hela cells were cotransfected with a reporter plasmid (pIFN-β-CAT) which contains

chloramphenicol acetyl transferase (CAT) gene under control of human IFN-β promoter and the individual plasmid expressing each viral protein. The IFN-β promoter is activated by transfection of another plasmid coding for human IRF3 (Interferon regulatory factor 3, the transcription factor responsible for activation of IFN-β transcription). All the plasmids coding for each viral protein were subjected to this reporter assay and the promoter inhibition/activation is monitored by measuring CAT activity. PRRSV non-structural protein 1 (Nsp1) was found to be the strongest repressors of the IFN-β promoter. In addition, Nsp2, Nsp4, Nsp11 and the nucleocapsid (N) protein exhibited measurable repression effect on IFN-β promoter. This implicates that multiple viral proteins may be involved in viral evasion of innate immune response in PRRSV infection. From the mechanistic viewpoint, we also checked the phosphorylation status of IRF3 in PRRSV infected MARC-145 cells.

Our results show that the IRF3 is being hyperphosphorylated upon viral infection. Future steps include checking any downstream impairment in the type I IFN signaling upon virus infection and fine mapping of the viral determinants responsible for inhibiting IFN-β induction.

2nd Place Poster Presentation

Relationship of CXCR1 genotypes with Responses to Experimental Challenge with *Streptococcus uberis*

A.A. Elliott*, S.P. Oliver, G.M. Pighetti

Department of Animal Science.
University of Tennessee, Knoxville, TN

Mastitis, an inflammation of the mammary gland, accounts for the largest loss in profit for dairy farmers. Observed differences in genetic susceptibility to mastitis between cows have led to ongoing research to identify why these differences occur. When bacteria enter the mammary gland, they first encounter macrophages and epithelial cells, which send messages to the rest of the body in the form of chemoattractants. One of the first targets of the chemoattractants, and therefore the first to arrive at the site of infection are the neutrophils. Our prior research has identified a polymorphism in the CXCR1 gene, a receptor for the chemoattractant interleukin-8 (IL-8) which is present on neutrophil surfaces. The polymorphism located at nucleotide position +777 induces an amino acid change from glutamine to histidine on the third intracellular loop of this G-protein linked receptor. This polymorphism has been associated with an increased incidence of mastitis, and research is ongoing to identify why these differences occur. This study evaluated local and systemic responses of cows with different genotypes at position +777 in the CXCR1 gene experimentally challenged with *Streptococcus uberis*. Holstein dairy cows with GG (n=7), GC (n=7), and CC (n=5) genotypes were challenged intramammarily with *S. uberis* strain UT888 and samples collected daily for 14 days. After the challenge, 57% of the cows with the GG genotype, 71% of cows with the GC genotype and 100% of cows with the CC genotype became infected (P=0.25). In order to assess differences among genetic backgrounds of infected cows, only those developing an infection were analyzed in the following data. Regardless of genotype, no differences were observed with respect to somatic cell count (P=0.64), bacterial growth among infected cows (P=0.58), rectal temperature (P=0.73), milk scores (P=0.41), and mammary scores (P=0.32). Cows with the CC genotype had a significantly lower number of circulating neutrophils at 6,270 cells/ml compared to 8,150 and 8,760 cells/ml for cows with the GG and GC genotypes respectively (P<0.04). However, 3 of the 5 non-infected cows also had white blood cell counts less than 6,800 cells/ml, suggesting the WBC count may not be the sole reason for greater susceptibility. Despite the small sample size for this type of study, the potential differences with respect to resistance to infection are promising. Once the infection occurs, there is little difference in response across genotypes, which suggests that early and potentially local responses provide the basis for altered disease resistance. Future studies involving a larger sample size will give a better indication of if and how cows with these genotypes differ in their ability to resist infection. Finding the reasons behind what makes some cows more genetically vulnerable to infection will provide an under-

standing which will help develop targeted strategies to prevent and treat mastitis infections.

Upcoming Events

The 95th Annual Meeting of the American Association of Immunologists (AAI) held in conjunction with Experimental Biology 2008, April 5-9, is in San Diego, CA. Information is available at http://www.aai.org/2008Meeting/Program.htm.

The AAI Veterinary Immunology Committee (AAI-VIC) and AAVI will host a joint symposium at the AAI meeting: **Comparative Biology of Dendritic Cells in Viral Infections.** Monday, April 7, 2008 beginning at 10:15 a.m., San Diego Convention Center, Room 33 A/B. Chairs: Christopher J. Davies, Utah State University (President, AAVI) and William T. Golde, USDA, ARS, Plum Island Animal Disease Center (Chair, AAI-VIC).

Speakers:
Simon M. Barratt-Boyes. University of Pittsburgh, *Dendritic cell dynamics in simian immuno-deficiency virus infection of monkeys*
Randy E. Sacco, National Animal Disease Center, *Modulation of neonatal ruminant pulmonary dendritic cells in respiratory syncytial virus infection*
Thomas M. Moran, Mount Sinai School of Medicine, *Kinetics of dendritic cell migration in mouse influenza virus infection*
William T. Golde, Plum Island Animal Disease Center, *Immune function of multiple dendritic cell subsets during FMDV infection in swine*

Comparative Models of Immune Responses, is April 9-11, 2008, at Lake Arrowhead, California. Co-Organizers: Bill Golde, USDA, ARS; Chuck Czuprynski, University of Wisconsin; and Tom Phillips, Western University of Health Sciences.

The meeting will include a keynote address by Max D. Cooper and five plenary sessions: (I) Biodefense: Immune Responses to High Impact Pathogens (Co-Chairs: David Woodland and Tom Phillips); (II) Platforms for Vaccine Development (Co-Chairs: Mark Estes and Roy Curtiss); (III) Comparative Immunogenetics of Immune Responses (Co-Chairs: Wendy Brown and John Butler); (IV) Comparative Models of Allergy, Nutrition, and Neoplasia (Co-Chairs: Chuck Czuprynski and Lynette Corbeil); and (V) Innate Responses and Immune Evasion (Co-Chairs: Bill Golde and Bruce Beutler). For more information check the meeting website: http://www.westernu.edu/cmir.

The Society for Leukocyte Biology Annual Meeting is November 6-8, 2008 in Denver, CO.

The Conference for Research Worker's in Animal Diseases is scheduled for December 7-9, 2008 at the Marriott (downtown magnificent mile) Chicago, IL.

5th International Veterinary Vaccines and Diagnostics Conference, will be in Madison, WI in July, 2009.

2007 AAVI Board and Business Meeting Minutes

AAVI Board Meeting
December 2, 2007
Los Angeles/Miami Room
Chicago Marriott Hotel

Attendees: Joan Lunney, Lorraine Sordillo, Eileen Thacker, Chris Davies, Doug Bannerman, Susan Eicher, Ron Schultz, Gina Pighetti, Carol Chitko-McKown

Absent: Paul Coussens, Subramaniam (Sri) Srikumaran, Jim Harp, Krishna Murthy.

1. Meeting was called to order by Joan Lunney at 8:02 AM.

2. Joan Lunney introduced new board members, Doug Bannerman as Vice President, Gina Pighetti as Secretary/Treasurer, Krishna Murthy as Board members and David Hurley and Randy

Sacco as new members of the Nominating Committee.

3. A motion was made to approve the agenda; it was seconded and accepted.

4. The minutes were approved as written by Eileen Thacker and corrected by Joan Lunney.

   Joan Lunney thanked Eileen for getting the organization back to fiscal soundness. Joan stated that Joan, Chris, and Doug will audit the books. The financial report was moved, seconded. Chris suggested specific corporate "dues". The financial report was approved based on a successful audit. (The audit was completed successfully after the meeting with one clarification in the December financial page).

5. **Old Business**

   a. **Committee Reports**

      1. **Student Awards Committee:** Carol Chitko-McKown reported that 4 awards were made last year at CRWAD. Seven awardees were also given travel funds for IVIS in Brazil. This year there will be 9 oral and 9 posters to be judged; for AAVI it was stated that the students should sign up for the immunology session. Susan Eicher, Carol Wyatt and Doug Bannerman will be judging oral presentations. Scott McVey Laura Miller, and Jishu Shi will judge posters. Most of the 8th IVIS travel awardees acknowledged AAVI and were also recognized at the meeting.

      2. **Nominations Committee:** Will Goff was not present. Chris reported that Jim Roth has agreed to be chair of nominations committee. Joan suggested people recommend nominations to the committee.

      3. **Fund Raising Committee:** Ron Schultz reported that Pfizer and Merial have paid corporate dues this year. Fort Dodge did not. Schering-Plough has agreed. Other potential companies BD, VMRD, ABI, Biorad, Fisher are possible sources of contributions. All people should keep Ron informed of possible donors.

      4. **Membership Committee:** Membership in AAVI is up, but Chris Davies and Gina Pighetti will work on further updating the list. There will be a membership table here for people to sign up or pay dues here at CRWAD.

      5. **Constitution/By-Laws Committee:** The outgoing President will update the Constitution/By-Laws for each year. Lorraine Sordillo has updated it for 2007 and it is ready for the membership to vote. This will be on the agenda each year. Joan said that if anyone has comments to let her know. The updated version will be voted on at the business meeting.

   b. **Website and maintenanc**e - Joan Lunney talked to Chris Minion and he will continue to run the website. He will help if Gina Pighetti wants to move it closer to her. Eileen Thacker will get him an Apple gift card for $100 (it was moved, seconded and approved). Chris Davies will contact Chris Minion about being able to access the website.

   c. **2007 AAVI/ACVM Symposium** (Jim Harp didn't make it from Iowa due to weather). Chris Davies will oversee it in his stead this year. Jim was bringing the projector, etc. It is unknown if all the speakers or Chris Chase will be here due to weather complications.

   d. **2008 AAVI/AAI Symposium** - A problem occurred with the AAI/VIC committee, but it was straightened out. Comparative Biology of Dendritic Cells in Viral Infections will be the topic for a joint symposium in San Diego on April 6, 2008. Chris Davies moved, it was then seconded and approved to take the speakers for the

AAVI/AAI symposium to lunch. This will be included in the by-laws.

e. John Butler is the recipient of the **DVI** award this year. Joan suggested that AAVI pay "reasonable" travel expenses. It was modified by Lorraine to change the cash award to a "travel award". It was moved that AAVI will pay the DVI $1,000 as either an honorarium or travel expenses. It was seconded and passed. It will start this year. This will also be included in the by-laws.

f. **Veterinary Immunology and Immunopathology (VII) report:** A report from Ken Plaxton was provided by Joan Lunney. It was encouraged that there not be too many special issues. Impact factor is going up. Electronic access to VII: It was decided that for 2008 online access is offered for $40/member that wants it. This decision was made by a fall 2007 e-mail poll of board members.

g. **USDA NRI travel grant for IVIS:** We were successful in obtaining the USDA CSREES monies. Gina will need to sign up early for Grants.gov. so the next grant can be easily submitted.

6. **New Business**

a. **2008 AAVI/ACVM Symposium topics:** Doug Bannerman will be in charge. Need topics for the session: innate immunity/ TLR's etc., antimicrobial peptides are possibilities. Will be working with Chris Chase and Scott McVey and will meet with them after the ACVM/AAVI board meetings to discuss potential topics for next years symposium. The topic, stressors could include innate immunity.

b. **2009 AAVI/AAI topics:** Jim Harp was not here so it will be discussed at a later date.

c. **Secretary/Treasurer transition.** Eileen and Gina are working to make the transition smooth. Joan asked if anyone wanted to archive the records. The new leadership will need to figure this out. Eileen will send all records to Gina.

d. **Luncheon/business meeting** will remain the same this year as last. Ron may have a sponsor for next year's Greek night.

e. **Credit card payments:** Gina found the best option is through Google checkout. Buyer enters the info and it saves it. You authorize Google to do a credit check. There is fraud protection with this program. If a person shares personal info, they are asked for permission. The site does store info and will share info on the people, but individuals can opt out. The credit card info would have to be put on Web page. If you are already registered, you could pay immediately, otherwise you would need to register. This program allows tax-exempt organizations to participate. There are no gateway fees, but there is a 2% fee – so it would be $1.30 for each transaction. Transactions can also be done internationally. It was moved and seconded that the treasurer would use Google credit. It was brought up whether to charge more to cover the cost, but it isn't worth pursuing. The motion was passed.

f. Joan reported on the **International Veterinary Immunology Symposium (IVIS)** in Brazil, which was in Ouro Preto. VII will have a special edition. The next meeting in 2010 will be Tokyo, Japan.

g. **IUIS/VIC**: Wayne Hein in New Zealand is the contact. Joan is the treasurer. Wayne is interested in attending regional meetings and could give a talk in the immunology session in the future.

h. **AAI/VIC** Joan Lunney discussed earlier. There is a new editor for JI and information was sent to the Board earlier. There was concern for veterinary immunology papers being fairly treated by JI. The new editor needs to be proactive. If there is a problem,

AAVI should be involved, so everyone should be aware.

i. **Newsletter editor:** Susan Eicher has agreed to be the new newsletter editor.

j. **Jeanne Burton memorial**: Thank you notes have not been done. The president should be in charge of writing them. We should be thinking about directed gifts. There is currently $400 that was given in her memory. At this point, the funds will be held until the future and hopefully the account will grow. A fund donation spot could be added to the membership form. The fund should be used to support student activities. It was moved that an AAVI student travel award in the memory of Jeanne Burton be awarded. The motion was amended to support student activities – not limited to CRWAD. Student activities including travel, posters and social activities in conjunction with AAVI. Fund raising for the **Jeanne Burton Memorial** is still underway with a projection for an event associated with 2008 CRWAD meeting.

k. **Standing Committee Chairs:**
Jim Roth – Nominating
Ron Schultz – Fund raising
Constitution and By-Laws – Joan Lunney
Student Awards – Carol Chitko-McKown
Membership – Jim Harp
Newsletter Editor – Susan Eicher

l. Potential for submitting a **USDA NRI General Travel Grant for the Vet Vaccine** meeting in Madison in 2009 was discussed. The USDA-NRI contact is Peter Johnson. There is a $10,000 limit for general travel requests. This would be directed for young investigators, grad students and post-doc. It was moved seconded and passed that a grant be put in. Carol, Ron and Gina will work on submitting the grant Eileen will send the grant from IVIS to Carol.

m. Additional New Business:
Joan Lunney – A gift card ($100) will be presented to Eileen for serving as Secretary-Treasurer.
The AAVI table at the CRWAD meeting will accept membership dues, have a copy of the by-laws, receipts, and will present information and accept monies for the Jeanne Burton memorial fund. The Board approved a Student membership fee of $20/year; this is not for postdocs. Verification by Dept. Chair will be required. A simple statement will be posted on the AAVI website as an example for students.

The meeting was adjourned at 10:43.

AAVI Business Meeting
Chicago Marriott Hotel
December 3, 2007

The meeting was called to order at 12:00

The officers of AAVI were introduced.

Joan Lunney acknowledged Doug Bannerman and Isis Mullarky for their organization of the Immunology session at this year's CRWAD meeting.

John Butler attended the meeting as DVI and was introduced.

Eileen Thacker acknowledged the increased membership and the great fund raising that have increased monies in our bank account.

Joan asked if members had other groups we should potentially be working with to increase our membership. Increased membership will enable us to send students to new and different meetings. Three poultry immunologists were on the ballot for officers this year and none were elected, this could be a problem.

The board thanked AAVI Secretary Eileen Thacker and voted to give her a gift card for her service.

Carol discussed student awards: There were 12 oral and 14 posters last year. AAVI gave out 4 awards last year, 2 for oral presentations and 2 for poster presentations. AAVI received a $10,000 grant from USDA-NRI that allowed 7 students to go to Brazil IVIS. Judges were named by Carol and consisted of both new and experienced people. People can contact Carol to volunteer for next year.

The Nomination Committee is ready to go. Chris Davies reported that the 2008 Nomination Committee is chaired by Jim Roth, who was not at the board meeting. Ron will continue as chair of the Fund Raising Committee. The Constitution and By-Laws will be reviewed by Joan Lunney. Carol Chitko-McKown will remain chair of student judging. Jim Harp will be the incoming president and Susan Eicher will be Newsletter editor.

Ron Schultz acknowledged Chris Chase for assisting in fund raising. He acknowledged all members are involved with fund raising. It would be helpful for anyone with contacts in industry to contact Ron or Chris Chase or Chris Davies for fund raising.

The 5th International Vaccine and Diagnostics Conference will be July 2009 in Madison. AAVI will apply next December to USDA-NRI for student travel. AAVI Greek dinner will be in the Parthenon this year.

Chris reported for the Membership Committee that this requires effort from all the members and encouraged members to invite new faculty to join. Gina is arranging credit card payment on the Web for dues. The student rate is $20 and $50 for regular members and an electronic subscription to VII will be an additional $40. VII will be charging more for electronic access so it is separated from the dues now.

The website will continue at Iowa State University maintained by Chris Minion. We gave him a gift to the Apple Store.

Yesterday's symposium went well. No reviews were requested from the presenters. Dr. John Butler's DVI talk will go to Animal Health Research Reviews.

AAVI/AAI symposium for 2008 will be Comparative Immunology of Dendritic Cells in Viral Infections. The AAI meeting will be followed by a special meeting on Comparative Models of Immune Responses at Lake Arrowhead. To try to organize buses, there is a need for people to register soon.

At the Board Meeting, it was decided that we will take speakers of AAVI/AAI Symposium to lunch. A mixer at AAI may be needed in the future to increase interest in membership.

The Constitution and By-Laws were re-done this past year, which hadn't been done for 5 years. Changes were made to up-date it. Members can go online and see the changes. We eliminated some committees and organized the current committee structure. The outgoing president will be in charge of reviewing the Constitution and By-Laws each year. This was announced in the August Newsletter. It was moved, seconded and the membership approved the 2007 changes in the constitution and by-laws.

Lynette Corbeil announced a meeting in Italy on Pasteurella. Information is on the CRWAD bulletin board.

Information about VII including time to publication, impact factor etc is available upon request.

New business:
AAVI/ACVM symposium topic for 2008 may be Stressors and Immunity.

Jim Harp will be in charge of the AAI/AAVI symposium next year.

The Secretary/Treasurer transition will occur over the next couple months. Google checkout will be used for credit cards. Memorial gift donations will also be available.

Joan asked if anyone wants to archive the AAVI records. Eileen will check with Iowa State University who has the ACVM archives.

Future business lunches were discussed. It was moved, seconded and voted that having a business luncheon prior to the DVI lecture would continue.

A new editor for Journal of Immunology, Jeremy M. Boss, was announced by AAI. He had a short notice on his goals for JI in the Dec. 2007 AAI Newsletter. It would be worth making note of those goals and assuring that veterinary immunology papers are being treated fairly by JI.

Chris Davies, the new president, thanked Joan Lunney for her service for AAVI and in veterinary immunology. Joan was presented with a plaque. He also thanked Eileen Thacker for being Secretary-Treasurer for the past 3 years. In closing Chris thanked AAVI for entrusting him with the position of President.

Chuck Czuprynski from the University of Wisconsin announced that he has a training grant and is looking for students.

The business meeting was adjourned at 12:55.

2008 AAVI Officers and Committee Members

2008 AAVI Officers and Board

President: Chris Davies, Utah State University, chris.davies@usu.edu
President Elect: James Harp, NADC, ARS, USDA, Jim.Harp@ars.usda.gov
Vice President: Doug Bannerman, BARC, ARS, USDA, douglas.bannerman@ars.usda.gov
Past President: Joan Lunney, BARC, ARS, USDA, Joan.Lunney@ars.usda.gov
Secretary-Treasurer: Gina Pighetti, University of Tennessee, pighetti@utk.edu
Members-at-Large:
Ron Schultz (2008), University of Wisconsin, schultzr@svm.vetmed.wisc.edu
Paul Coussens (2009), Michigan State University, coussens@msu.edu
Subramaniam (Sri) Srikumaran (2010), Washington State University, ssrikumaran@vetmed.wsu.edu
Krishna K. Murthy (2011), Southwest Foundation for Biomedical Research, kmurthy@sfbr.org

Nomination Committee (Elected by membership):

Jim Roth, Chairperson (2008), Iowa State University
jaroth@iastate.edu
Phil Elzer (2008), Louisiana State University
pelzer@agctr.lsu.edu
Pat Shewen (2009), University of Guelph
pshewen@uoguelph.ca
Chris Chase (2009), South Dakota State University
christopher_chase@sdstate.edu
David Hurley (2010), University of Georgia
dhurley@vet.uga.edu
Randy Sacco (2010), NADC, ARS, USDA, Randy.Sacco@ARS.USDA.GOV)

Newsletter Editor:

Susan Eicher, LBRU, ARS, USDA, Susan.Eicher@ars.usda.gov

Fund Raising Committee:

Ron Schultz, Chairperson, University of Wisconsin
schultzr@SVM.VETMED.WISC.EDU
Chris Chase, South Dakota State University
christopher_chase@sdstate.edu
Paul Coussens, Michigan State University
coussens@msu.edu
Elizabeth Davis, Kansas State University
edavis@vet.ksu.edu
Lorraine Sordillo, Michigan State University
sordillo@msu.edu
Dennis Foss, Pfizer Animal Health
dennis_l_foss@pfizer.com

Constitution/By-Laws Committee:

Joan Lunney, Chairperson, BARC, ARS, USDA,
Joan.Lunney@ars.usda.gov
James Harp, NADC, ARS, USDA
Jim.Harp@ars.usda.gov
Chuck Czuprynski, University of Wisconsin
czuprync@svm.vetmed.wisc.edu

Student Awards Committee:

Carol Chitko-McKown, Chairperson, USMARC, ARS, USDA
carol.chitkomckown@ars.usda.gov
2007 Judges were Doug Bannerman, Susan Eicher, Scott McVey, Laura Miller, Jishu Shi, and Carol Wyatt.

Membership Committee:

James Harp, Chairperson, NADC, ARS, USDA,
Jim.Harp@ars.usda.gov
Chris Davies, Utah State University,
chris.davies@usu.edu
Joan Lunney, BARC, ARS, USDA
Joan.Lunney@ars.usda.gov
Paul Coussens, Michigan State University
coussens@msu.edu
Cynthia Baldwin, University of Massachusetts
cbaldwin@vasci.umass.edu

US-VETERINARY IMMUNE REAGENT NETWORK

WWW.VETIMM.ORG

Updated 2-6-08

TABLE OF CONTENTS

I. CHEMOKINES & CYTOKINES

- In most cases the genes described were cloned and sequenced by US-VIRN P.D.'s. Whole sequence includes signal/leader sequence.
- All these genes are available for distribution upon request and we are investigating feasibility of deposition in gene banks.
- All sequences have been/will be deposited in Genbank.
- Kingfisher (KF) biotech will be expressing these in Pichia and sending to the various species coordinators for biotesting.
- The bioactive proteins will be commercially available and a limited amount directly to scientists.
- A selected number (30-40 molecules) will be used for producing mAbs by US-VIRN.

CATFISH											
	WHOLE CODING SEQUENCE WITH SIGNAL/LEADER				**GENE SEQUENCE FOR MATURE EXPRESSED PROTEIN**			**EXPRESSION PROGRESS**			
Gene	**Clone #**	**FASTA File**	**US-VIRN GenBank #**	**Glycerol Stock**	**Clone #**	**FASTA File**	**Glycerol Stock**	**Stage**	**Transformed in expression vector**	**In yeast at KF**	**Bioactivity affirmed**
IFN-γ with signal sequence	√	√	DQ124250	UMiss labs				KF primers made			
IFN-γ without signal sequence	√			UMiss labs				KF primers made			
Type I IFN	√	√	AY847295	UMiss labs							

EQUINE											
	WHOLE CODING SEQUENCE WITH SIGNAL/LEADER				GENE SEQUENCE FOR MATURE EXPRESSED PROTEIN			EXPRESSION PROGRESS			
Gene	**clone #**	**FASTA**	**US-VIRN GenBank #**	**Glycerol stock**	**Clone #**	**FASTA**	**Glycerol stock**	**Stage**	**Transformed in expression vector**	**In yeast at KF**	Bioactivity affirmed
CCL2	TH-295	√	EU438774	√	TH-281	√	√	Sent to KF	√		
CCL3	TH-337	√	EU438775	√	TH-343	√	√	Sent to KF			
CCL5	TH-367	√	Submitted	√				Primer re-design			
CCL11								need tissue			
CXCL9	TH-351	√	EU438776	√	TH-341	√	√	Sent to KF		2/08	
CXCL10	TH-345	√	EU438777	√	TH-357	√	√	Sent to KF		2/08	
GM-CSF	TH308	√	EU438778	√	RN-027	√	√	Sent to KF	√		
IFN-γ	Wagner BW143	√	U04050	√	TH-201	√	√	Sent to KF	√		
IFN-α1	PCR product				TH-278	√	√	Sent to KF	√		
IL-1β	DT-111	√	EU438767	√	RN-003	√	√	Sent to KF	√		
IL-2	DT-113	√	EU438768	√	RN-014	√	√	Sent to KF	√		
IL-4	DT-117	√	EU438769	√	RN-005	√	√	Sent to KF	√	√	
IL-5	PCR product							Primers designed			
IL-6	DT-122	√	EU438770	√	RN-016	√	√	Sent to KF	√		
IL-10	DT-126	√	EU438771								
IL-12 p35	Primer re-design										
IL-12 p40	Primer re-design										
IL-13	Wagner BW133	√	EF645663	√				Primer design			
IL-15	TH-379	√	Submitted	√	RN-029	√	√	Sent to KF	√		
IL-17	PCR product				RN-020	√	√	Sent to KF	√		
IL-18	DT-001	√	EU438772	√	RN-011	√	√	Sent to KF	√	√	
IL-23	TH-287	√	EU438773	√	TH-279	√	√	Sent to KF	√		
TGF-β	Wagner BW106	√	X99438	√	TH-273	√	√	Sent to KF	√		
TNF-α	TH322	√	EU438779	√	TH-285	√	√	Sent to KF	√		

POULTRY - CHICKEN										
	WHOLE CODING SEQUENCE WITH SIGNAL/LEADER			GENE SEQUENCE FOR MATURE EXPRESSED PROTEIN			EXPRESSION PROGRESS			
Gene	**Lillehoj Clone #**	**US-VIRN GenBank #**	**Glycerol stock**	**US-VIRN Clone #**	**FASTA**	**Glycerol stock**	**Stage**	**Transformed in expression vector**	**In yeast at KF**	**Bioactivity affirmed**
CCL4	H112	NM001030360	Lillehoj lab	DT-362	√	√	Sent to KF	√	2/08	
CCL20	H113	NM204438	Lillehoj lab	DT-358	√	√	Sent to KF	√		
IFN-γ	H029	AH009942	Lillehoj lab	TH-264	√	√	Sent to KF	√		
IL-1β	H242	Y15006	Lillehoj lab	TH-259	√	√	Sent to KF	√		
IL-2	H030	AF017645	Lillehoj lab	TH-265	√	√	Sent to KF	√		
IL-4	H107	NM 001007079	Lillehoj lab	DT-352	√	√	Sent to KF	√	2/08	
IL-10	H108	NM 001004414	Lillehoj lab	RN-034	√	√	Sent to KF	√	√	
IL-12p35	H109	NM213588	Lillehoj lab	DT-650	√	√	Sent to KF		2/08	
IL-12p40	H110	Ay262752	Lillehoj lab	DT-654	√	√	Sent to KF			
IL-15	H238	NM 204571	Lillehoj lab	DT-363	√	√	Sent to KF	√		
IL-16	H033	AJ508678	Lillehoj lab	DT-606	√	√	Sent to KF			
IL-17	H032	AJ493595	Lillehoj lab	TH-262	√	√	Sent to KF	√		
IL-17D	H008	Ef570583	Lillehoj lab	DT-660	√	√	Sent to KF			
IL-18	H020	AJ277865	Lillehoj lab	DT-325	√	√	Sent to KF	√		
LITAF	H103	AY765397	Lillehoj lab	TH-265	√	√	Sent to KF	√		
Lymphotactin	H026	AF006742	Lillehoj lab	DT-341	√	√	Sent to KF	√		
MIF	H013	M95776	Lillehoj lab	DT-665	√	√	Sent to KF			
TNFSF15 (TL1A)	H105	NM001024578	Lillehoj lab	TH-269	√	√	Sent to KF	√		

RUMINANTS-BOVINE											
	WHOLE CODING SEQUENCE WITH SIGNAL/LEADER				GENE SEQUENCE FOR MATURE EXPRESSED PROTEIN			EXPRESSION PROGRESS			
Gene	Clone #	FASTA File	US-VIRN GenBank #	Glycerol Stock	Clone #	FASTA File	Glycerol Stock	Stage	Transformed in expression vector	In yeast at KF	Bioactivity affirmed
CCL2	TH-33	√	EU276069	√	TH-80	√	√	Sent to KF	√	2/08	
CCL5	TH-21	√	EU276060	√	TH-76	√	√	Sent to KF	√	2/08	
CCL11	TH-333	√	Submitted	√	TH-172	√	√	Sent to KF	√		
CXCL9	TH-6	√	EU276061	√	TH-152	√	√	Sent to KF	√	√	
CXCL10	TH-13	√	EU276062	√	TH-88	√	√	Sent to KF	√	√	
CXCL11	TH-11	√	EU276063	√	TH-101	√	√	Sent to KF	√	2/08	
IFN-γ	TH-30	√	EU276066	√	TH-72	√	√	Sent to KF	√		
IFN-α	TH-1	√	EU276064	√	TH-148	√	√	Sent to KF	√		
IFN-β	TH-113	√	EU276065	√	TH-141	√	√	Sent to KF	√		
IL-1β	TH-94	√	EU276067	√	TH-94	√	√	Sent to KF	√		
IL-2	TH-36	√	EU276068	√	TH-129	√	√	Sent to KF	√		
IL-4	TH-132	√	EU276069	√	TH-132	√	√	Sent to KF	√		
IL-5	TH-185	√	EU276070	√	TH-177	√	√	Sent to KF	√		
IL-6	TH-135	√	EU276071	√	TH-135	√	√	Sent to KF	√		
IL-7	TH-114	√	EU276072	√	TH-114	√	√	Sent to KF	√		
IL-8	TH-18	√	EU276073	√	TH-84	√	√	Sent to KF	√		
IL-10	TH-104	√	EU276074	√	TH-160	√	√	Sent to KF	√		
IL-12p35	TH-180	√	EU276075	√	TH-165	√	√	Sent to KF	√		
Il-12p40	TH-43	√	EU276076	√	TH-139	√	√	Sent to KF	√		
IL-13	TH-90	√	EU276077	√	TH-90	√	√	Sent to KF	√		
IL-15	VML94	√	Submitted	√	TH-348	√	√	Sent to KF			
IL-17	VLM96	√	Submitted	√	TH-121	√	√	Sent to KF	√		
IL-18	TH-118	√	EU276078	√	TH-118	√	√	Sent to KF	√		
IL-23	CC13	√	Submitted		TH-126	√	√	Sent to KF	√		
TNF-α	TH-96	√	EU276079	√	TH-96	√	√	Sent to KF	√		

SWINE											
	WHOLE CODING SEQUENCE WITH SIGNAL/LEADER				GENE SEQUENCE FOR MATURE EXPRESSED PROTEIN			EXPRESSION PROGRESS			
Gene	Clone #	FASTA File	US-VIRN GenBank #	Glycerol Stock	Clone #	FASTA File	Glycerol Stock	Stage	Transformed in expression vector	In yeast at KF	Bioactivity affirmed
CCL2	DT-304	√	Submitted	√	DT-616	√	√	Sent to KF	√	2/08	
CCL3L1	DT-401	√	EU364893	√	DT-639	√	√	Sent to KF			
CCL4	DT-426	√	EU364894	√	PCR product						
CCL5	PCR product				DT-515	√	√	Sent to KF	√	2/08	
CXCL9	DT-305	√	EU36897	√	DT421	√	√	Sent to KF	√		
CXCL10	DT-308	√	EU364898	√	TH-249	√	√	Sent to KF	√	√	
CXCL11	PCR product			√	DT-432	√	√	Sent to KF	√		
IL-7	DT-300	√	EU364895	√	DT-413	√	√	Sent to KF	√		
IL-13	DT-321	√	Submitted	√	TH-253	√	√	Sent to KF	√		
IL-15	Zarlenga	√	NM 214390	n.a.	DT-228	√	√	Sent to KF	√		
IFN-α	DT-316	√	EU364896	√	DT-440	√	√	Sent to KF			
IFN-β1	PCR product				DT-627	√	√	Sent to KF			
TNF-α	DT-211	√	Submitted	√	DT-624	√	√	Sent to KF			

TROUT											
	WHOLE CODING SEQUENCE WITH SIGNAL/LEADER				GENE SEQUENCE FOR MATURE EXPRESSED PROTEIN			EXPRESSION PROGRESS			
Gene	**Clone #**	**FASTA File**	**US-VIRN GenBank #**	**Glycerol Stock**	**Clone #**	**FASTA File**	**Glycerol Stock**	**Stage**	**Transformed in expression vector**	**In yeast at KF**	**Bioactivity affirmed**
IFN-a	√							John Hansen cloned			
IFN-a	√							John Hansen cloned			
IFNγ	√							John Hansen cloned			

II. CELL SURFACE MOLECULES

- These are being transfected into CHO cells at Cornell by Bettina Wagner except where indicated * then the proteins and monoclonal antibody are being or have been produced in species lab
- Gene sequences have been/will be deposited into GenBank
- Most genes are available upon request
- All the proteins listed here will be used for producing monoclonal antibodies
- Transfected cell lines will also be available for testing other potential cross-reactive monoclonal antibodies
- The hybridoma cell lines that produce the Monoclonal antibody will be deposited in cell banks for distribution upon request and distributed to a limited number of scientists directly by Network members; the monoclonal antibody they produce will available through commercial companies

CATFISH						
Molecule	**Full length cDNA with signal sequence**	**GenBank #**	**Gene cloned into expression system**	**Stable transfectant**	**Protein being produced/ purified**	**Monoclonal antibody status**
TCR α	√		Yes	Yes	Yes	In progress
TCR β	√		Yes	Yes		
TCR δ	√		Yes	Yes		
TCR γ	√		Yes	Yes	Yes	In progress
IgD	√		Yes*	Yes*	Yes*	Made mAbs but only IgMs were obtained*

EQUINE					
Molecule	**Full length cDNA with signal sequence**	**Gene cloned into expression system**	**Stable transfectant**	**Protein being produced/ purified**	**Monoclonal antibody status**
CD40	√	Yes	Yes	Yes	Fusion ongoing*
CD23	√	Yes	Yes	Yes	
CD25 (IL-2Rα)	√	Yes	Yes	Yes	Fusion ongoing
CD28	√	Yes	Yes		
FceRIα	√	Yes	Yes	Yes	Fusion ongoing
TCR α	√ (C-region)	Yes			
TCR ß					
TCR γ	√ (C-region)				
TCR δ	√ (C-region)				
IgD	√	Yes			
IgG2	√				
IgG3	√				
IgG6	√	Yes	Yes	Yes	Made mAb*
CD16	√				

Tbd					
Tbd					
Tbd					

POULTRY- CHICKEN					
Molecule	**Full length cDNA with signal sequence**	**Gene cloned into expression system**	**Stable transfectant**	**Protein being produced/ purified**	**Monoclonal antibody status**
IL-2Rα	√	Yes			
IL-21R	Primers made				
CXCR4	√	Yes			
CD80	Lillehoj – in progress				
CD83 or CD86	Lillehoj – in progress				
CTLA4	√	Yes*	Yes*		

RUMINANTS - CATTLE					
Molecule	**Full length cDNA with signal sequence**	**Gene cloned into expression system**	**Stable transfectant**	**Protein being produced/ purified**	**Monoclonal antibody status**
TCR δ	√	Yes	Yes	Yes	
TCR γ	√	Yes	Yes	Yes	ongoing
TCR α	√				
TCR β	√				
IL-23R	√	Yes			
IL-10R	√	Yes			
CCR7	√	Yes			

SWINE					
Molecule	**Full length cDNA with signal sequence**	**Gene cloned into expression system**	**Stable transfectant**	**Protein being produced/ purified**	**Monoclonal antibody status**
TCRγ	EU364901				
TCR α	EU364899	Yes	Yes	Yes	Mice immunized
TCR β	EU364900	Yes	Yes		
IL-4Rα	√	Yes	Yes		
IL-13Rα1	√	Yes	Yes		
CD45RO	Not needed	Not needed	peptide	Not needed	Mice immunized
IGSF2					
IL-7R					
CCR7					
CXCR3					

TROUT					
Molecule	**Full length cDNA with signal sequence**	**Gene cloned into expression system**	**Stable transfectant**	**Protein being produced/ purified**	**Monoclonal antibody status**
P56 lck	√	Yes*	Yes*	Yes*	Made mAbs*
Pax5	√	Yes*	Yes*	Yes*	Made mAbs*
Blimp	√	Yes*	Yes*	Yes*	Made mAbs*
TCR α	√	Pending			
TCR β	√	Pending			
TCR γ	√	Pending			
CD3	√	Yes*	Yes*	Yes*	
CD4	√	Yes*	Yes*		
CD4-REL	√	Yes*	Yes*		
CD8	√	Yes*	Yes*	Yes*	
CD28	√	Yes*	Yes*		
CTLA4	√	Yes*	Yes*		

*For all tables this indicates made in “species lab”

III. MOLECULES FOR MONOCLONAL ANTIBODY PRODUCTION

- The cell surface molecules being expressed at Cornell in the Wagner Lab (see table above) will all be used for producing monoclonal antibodies
- In addition, there are plans to make monoclonal antibody against about 30-40 cytokines or chemokines for different species as noted in the Tables above once they have shown bioactivity
- Some species labs are producing monoclonal antibody against molecules in their own labs as well.

Grant goal is 12 monoclonal antibodies per species	SPECIES					
	HORSE	**BOVINE**	**CHICKEN**	**SWINE**	**CATFISH**	**TROUT**
#1	CD16	TCR α	IL-2Rα	TCR α	TCR α	TCR α
#2	CD23	TCR ß	IL-21R	TCR β	TCR β	TCR β
#3	CD28	TCR γ	CXCR4	CD45RO	TCR δ	TCR γ
#4	CD40	TCR δ	CD80	IL-4Rα	TCR γ	IFNγ
#5	TCR α	IL-23R	CD83 or CD86	IL-13Rα1	IgD	IFNα
#6	TCR ß	IL-10R	IL-12 p40	CXCL10	IFNγ	Cyto/chemokine
#7	TCR γ	CCR7	IL-12 p35	CCL5	Cyto/chemokine	Cyto/chemokine
#8	TCR δ	Cyto/chemokine	IL-17	CCL2	Cyto/chemokine	Cyto/chemokine
#9	Cyto/chemokines*	Cyto/chemokine	IL-17D	CXCL11	Cyto/chemokine	Cyto/chemokine
#10	Cyto/chemokine	Cyto/chemokine	IL-16	IL15	Cyto/chemokine	Cyto/chemokine
#11	Cyto/chemokine	Cyto/chemokine	IL-10	IL13	Cyto/chemokine	Cyto/chemokine
#12	Cyto/chemokine	Cyto/chemokine	IL-4	IL7	Cyto/chemokine	Cyto/chemokine
Additional monoclonal antibodies beyond minimum proposed						
#13	IgG6			IFNα	IgD	P56 lck
#**14**	CD25			TNF α		Pax5
#15	FceRIα			IFN□1		Blimp
#16	*Tbd*			CCL4		CD8
#17	*Tbd*			CCL3L1		CD28
#18				IGSF2		CTLA4
#19				IL-7R		CD3
#20				CCR7		CD4
#21				CXCR3		CD4-REL

*****Cyto/chemokine: cytokines or chemokines; order of priority for antibody production is dependent on Pichia expression and bioactivity.
***Tbd: To be determined*