

## REPORT OF THE COMMITTEE ON BIOLOGICS AND BIOTECHNOLOGY

Chair: Bob Pitts, Athens, GA

Vice Chair: Vacant

Gary A. Anderson, KS; Joan M. Arnoldi, WI; Charles A. Baldwin, GA; Yung Fu Chang, NY; James J. England, ID; James Evermann, WA; William H. Fales, MO; Robert W. Fulton, OK; Ted Girshick, CT; Keith N. Haffer, SD; Larry L. Hawkins, MO; Ruud G. Hein, DE; Richard E. Hill, IA; Joseph N. Huff, CO; Majon Huff, CO; Hiram N. Lasher, DE; Lloyd H. Lauerma, WA; Mr. John C. Lawrence, ME; Randall L. Levings, IA; David Marshall, NC; Robert E. Pitts, WV; Nasir Hussain Shah, PAK ; Deoki N. Tripathy, IL; Mr. Bob Tully, KS; Ellen Wilson, CA; James Wolfram, FL.

Committee Members in Attendance: Sarah Chalangan, MO; William Fales, MO; Joe Huff, CO; Bob Pitts, GA; Ellen Wilson, CA; Jim Wolfram, FL

The Committee met on October 12, 2009 at the Town and Country Hotel, San Diego, CA, from 7:00 p.m. to 10:15 p.m.. There were 6 members and 14 guests present. Chair Bob Pitts called the meeting to order. He introduced himself as a long time member of the committee, one involved in vaccine research, production and testing for 34 years, Vice President of Quality Assurance and Regulatory Affairs at Bioniche Animal Health USA, Inc. for 18 years and an individual committed to quality animal health. The Committee had everyone introduce themselves. Chair Pitts expressed pleasure at the turnout and interest. The Vice-Chairman position is vacant and applicants were encouraged. The committee Mission Statement was reviewed: "The purpose of the Biologics and Biotechnology Committee is to monitor 1) new development in veterinary biologics, 2) regulation of the manufacture, distribution and use of veterinary biologics, and 3) needs of the livestock industries for new biological products. The Committee has the responsibility of keeping abreast and advising USAHA of new biotechnology, products and regulations that may have profound economic implications on animal health. Further, the Committee provides a forum to focus on issues and developments in the field of biotechnology that are designed to provide protection to man, animals and the environment.

The Chair recognized the participation by key USDA personnel and thanked them for accepting speaker roles. Dr. Hill and Dr. Whipple had been scheduled as speakers but both were called back to Ames unexpectedly. Dr. Rippke and Dr. Kehrl, respectively, presented for them.

### **APHIS-VS-Center for Veterinary Biologics (CVB) Program Updates and Issue Discussion**

Dr. Byron Rippke, Director of CVB-Licensing and Policy Development (LPD)

Dr. Rippke told the members about the successful move into the new facilities at the National Centers for Animal Health (NCAH). This \$460 million project is proceeding well. The Combined Service Plan involves 286 support personnel with the goal of providing support activities to CVB, NACS, ARS, and NVSL. It was noted that Operational expenses (utilities, etc.) were again not included in the budget. The electric bill was over 5 million dollars. The compliance in the new facilities with the Select Agent Program is an important and big project.

He showed the current APHIS organization chart that now includes an informational section with John Picanso as the Chief Information Officer reporting to Dr. Clifford.

The Strategic Vision for 2015 plan was discussed and the formation of 4 working groups to help implement it. The One Health Group is formed and active and the other 3 are in various stages of formation. It is their intention to synthesize their recommendations to assure they meet regulatory, budget and technological requirements. CVB is seeking stakeholders to provide input to this plan.

With changing disease situations, CVB is adjusting their approach to such diseases as Bovine Tuberculosis and Bovine Brucellosis. Position papers are available on their web site.

In 2008 CVB asked for \$16.6 million but was given \$12.63 million. In 2009, they asked for \$M 16.92 and got \$M 14.51. In 2010 they asked for \$M 17.325. CVB continues to handle 4,462 submissions, issue 55 new licenses released 13,868 serials of product with limited personnel and limited budget. User Fees are being considered and may be implemented in the next few years. The vacancy situation is:

Reviewers - 5 vacancies out of 17 positions

- Specialists – 8 out of 16
- Epidemiologists – 1 out of 2

- Statisticians – 1 out of 5
- Lab VMO/Micro – 8 out of 18
- Techs – 8 out of 28
- Section Leaders – 1 out of 10
- Ast/Assoc Dir. – 1 out of 2
- Support Staff – 5 out of 35
- Info Management – 7 out of 35

Influenza H1N1 seed lots were made and distributed to vaccine manufactures in a well thought-out activity to expedite any new vaccines. An expedited Conditional License approach is also available.

CVB has also been active in a coalition concerning pre-harvest interventions with E. coli O157 in cattle. A Conditional License was issued this year to one manufacture.

Additional issues and activities include Pharmacovigilance, *In vitro* policies and guidance documents, planned participation for Viral Safety and Extraneous Agent Testing For Veterinary Vaccines in France, October 25-28, 2009, and participation in a Potency Testing – In vivo Int'l Workshop in Germany, December 2010.

In a cost and time-saving move, the annual Public Meeting scheduled in the spring of 2010 was cancelled.

### **Creating the New NADC: A Progress Report**

Dr. Marcus Kehrli, Jr., NADC, Ames, IA, Research Leader, Virus and Prion Diseases

Dr. Kehrli presented this for Kurt Zuelke, DVM, PhD, NADC Director, Diana L. Whipple, NADC Deputy Director, who were unable to attend.

This past year has been quite eventful at NADC with the successful relocation from our 48-year-old research facilities into the new Combined Laboratory Facility of the National Centers for Animal Health that now co-houses NADC of the Agricultural Research Service, and CVB and NVSL of APHIS. The move was 95% completed the first week of September. Plans were put into place and successfully executed to maintain research efforts at the NADC with minimal interruption. The new research laboratories are now operational and they work very well to support our research programs. Our focus now is to strategically position NADC's research capacity to address the most critical animal health research needs of the U.S. livestock industries. We continue to maintain our science-based, customer-focused, and forward-looking research capacity that will enable NADC to diversify and increase our funding base and synergize opportunities in the new facilities with our APHIS colleagues. Our research mission will continue to be aligned with the USDA priorities of 1) International food security by maximizing US animal health to assure international market access; 2) Sustainable opportunities for economic growth and prosperity for rural communities and producers by developing effective disease detection and control technologies for cost-effective livestock production; 3) Assure a safe and wholesome food supply by decreasing pathogen prevalence among livestock and the impact of food borne pathogens; 4) Protecting animal and human health through cutting edge research to deliver next generation diagnostics, vaccines and other novel disease intervention strategies. This past year we also made strategic decisions to reduce scientific staff to address pressing financial issues associated with operation of new and old research facilities and as part of this, we reorganized the NADC scientific staff around key scientific themes into the following 4 research units: 1) Ruminant Diseases and Immunology Unit, 2) Infectious Bacterial Diseases Unit, 3) Food Safety and Enteric Pathogens Unit, 4) Virus and Prion Disease Unit. Our business plan for the future is to re-grow our critical mass of scientists as operational costs decrease with the decommissioning and demolition of our old facilities. A couple research highlights for this past year include the research conducted with the novel 2009 (A/H1N1) pandemic virus that we conducted in pigs that has demonstrated the pathogenesis of the pandemic virus in pigs and that pork from pigs that recover from this virus is safe to handle and eat as the virus is only isolated from respiratory tract tissues during the acute stages of the disease and pigs quickly recover from the infection and no longer shed virus. We also were able to establish the efficacy of commercial vaccines against this novel virus should the virus become established in U.S. pigs. In addition, this past year NADC scientists organized and participated in the 2009 ARS Metagenomics Conference.

### **Influenza Research Update**

Dr. Marcus Kehrli, Jr., NADC, Ames, IA, Research Leader, Virus and Prion Diseases

The following researchers were recognized as part of the Influenza team: Amy L. Vincent, DVM, PhD, Alessio Lorusso, DVM, PhD, Janice Ciacci-Zanella, DVM, PhD, Eraldo Zanella, DVM, PhD, Kelly A. Lager, DVM, PhD, Kay S. Faaberg, PhD, Marcus E. Kehrli, Jr., DVM, PhD\* Swine and Prion Diseases Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA.

Soon after the emergence of the H1N1 virus in April 2009, ARS scientists at the National Animal Disease Center in Ames, Iowa, began research using virus samples provided by the Centers for Disease Control and Prevention (CDC). Our immediate attention went to developing 2 differential diagnostic tests (one RT-PCR and one gel-based RFLP) based on the novel matrix gene present in the pandemic virus, this work was completed the same day (01May09) that we began inoculating pigs in a pilot pathogenesis study. We also designed a larger pathogenesis/transmission study that began shortly thereafter. The first pig studies were designed to evaluate whether the novel 2009 (A/H1N1) pandemic virus would infect, cause disease in and transmit between pigs; these two separate studies quickly answered this question and the pandemic virus strains tested were confirmed to be pathogenic in and transmissible between pigs. As part of these pathogenesis studies, it was confirmed that 2009 (A/H1N1) pandemic influenza virus was only isolated from tissues associated with the respiratory tract in acutely infected pigs and that pigs quickly recover from the infection and the virus was no longer able to be isolated. Next our focus was whether current U.S. H1N1 swine influenza vaccines can protect pigs from infection with the 2009 H1N1 influenza virus circulating in people. Our research also evaluated whether pre-existing titers in pigs previously infected with endemic H1N1 swine influenza viruses circulating in the U.S. pigs could protect against the 2009 (A/H1N1) pandemic influenza virus and it was found that pigs that had recovered from a circulating endemic swine influenza virus appear to have substantial cross-protection against subsequent challenge with the pandemic virus. Finally, 3 commercial vaccines were selected for efficacy testing against the pandemic virus based on serological cross-reactivity of vaccine antisera in a hemagglutination inhibition assay using 2009 A/H1N1 influenza viruses isolated from persons in California, New York, and Mexico. Results showed that in spite of limited cross reactivity against the new 2009 A/H1N1 influenza viruses the 3 vaccines tested each provided significant protection against lung lesions in pigs challenged with a 2009 (A/H1N1) pandemic influenza virus. The most optimal protection was seen with an inactivated vaccine made from the homologous pandemic virus. Importantly, none of the vaccines tested caused disease enhancement in the lungs as is sometimes observed when the challenge virus is a mismatch with the vaccine virus strain. We have also tested experimental MLV vaccines and will continue research to develop new vaccines that afford the best degree of heterologous protection possible.

### **Nanotechnology-based detection and diagnostic tools for livestock pathogens**

Dr. John Neil, National Animal Disease Center (NADC), Ames, IA, Microbiologist in Ruminant Diseases

Historically, cell cultures have been used as an integral part of diagnostic tests for livestock pathogens. These tests include serum neutralization and virus isolation. However, cell culture is cumbersome, time consuming, labor intensive and expensive. Other assay platforms have been developed to overcome these shortfalls. ELISAs and other solid phase diagnostics have seen wide use but still lack sufficient sensitivity in some applications.

New readout technologies have allowed the development of rapid and extremely sensitive diagnostic assays that measure antibody/antigen interactions. One such platform, surface enhanced raman scattering (SERS) has been shown to be low cost, rapid, sensitive and extremely reproducible. SERS is based on gold nanoparticles that have been conjugated to a raman reporter and a monoclonal antibody that is specific for the pathogen target. This technology has sensitivity to at least femtomolar concentrations of analyte. Because the excitation wavelength of the laser that used in the system is dependent on the test substrate and not on the analyte, only a single laser is needed. The finished assays are stable over long periods of time because the reactions are not sensitive to humidity, quenchers or to fading. Thus they can be archived for long periods of time without loss of signal should they need to be analyzed again.

In proof of concept experiments, 2 animal viruses were used to test the system, feline calicivirus (FCV) and porcine parvovirus (PPV). These were chosen because they are grown to high titers and monoclonal antibodies were readily available. The first experiments involved the attachment of the monoclonal antibodies to the gold substrate and then analyzing capture of FCV to the substrate by atomic force microscopy. These experiment showed that dilution of virus resulted in less virus captured as expected. The same results were obtained using the SERS gold nanoparticles where declining signal

was observed with dilution of the virus. This initial SERS test detected down to  $1 \times 10^6$  TCID<sub>50</sub>/ml of FCV. Again, similar results were obtained using PPV as the analyte.

In experiments to increase sensitivity of the SERS technology, the size of the assay address was tested. In these experiments, it was found that the smaller the address used, the greater the sensitivity. When an address of 2 mm was used the limit of detection of PPV was  $1 \times 10^4$  TCID<sub>50</sub>/ml. However when an address of 0.2 mm was used, the level of detection dropped to 45 TCID<sub>50</sub>/ml. The laser used for the detection of captured nanoparticles had a beam diameter of 0.2 mm. By analyzing the entire address rather than a small portion, the sensitivity was greatly increased.

The shape of the nanoparticles was also tested. It was found that changing the shape from spherical (used in the experiments described above) to cubic resulted in a 300x increase in level of detection. Similarly, rotation of the gold-coated substrate in the solution to be analyzed resulted in greater sensitivity and a significant decrease in time necessary to conduct the assay.

Research is ongoing to develop and improve the SERS technology and adapt it to use with more relevant livestock pathogens. However, it is possible that assays will soon be developed that will find utility in veterinary diagnostics.

### **Brucellosis in the U.S. and Ongoing Vaccine Research**

Steven Olsen, National Animal Disease Center (NADC), Ames, IA, Veterinary Medical Officer, Bacterial Diseases

Brucellosis is a series zoonotic pathogen which continues to persist in wildlife reservoirs despite being essentially removed from domestic livestock. There is a need to develop new vaccines and technologies to address brucellosis in wildlife reservoirs. We have evaluated various *Brucella* vaccines and vaccine delivery systems in domestic wildlife and demonstrated efficacy in targeted species. However, because of cost and limitations in delivering vaccines to wildlife, the most efficacious vaccines that can most efficiently delivered are needed. Our project continues to work to develop new and improved vaccines and delivery methods. We are also currently characterizing pathogenesis and serology after challenge of cattle with *B. suis*. Although the project is ongoing, preliminary data suggests that *B. suis* is not abortigenic in cattle and that colonization in cattle is reduced as compared to *B. abortus* experimental challenge.

### **Committee Business:**

Last year's Resolution Number 32 was reviewed. This resolution asked USDA to request and utilize Operational Expenses for the National Centers for Animal Health that would not impact program funding. The response stated the money was requested in the 2009 President's Budget but was not realized by Congress in the final 2009 Budget. They will be requested again. It was the consensus of all committee members that the operational expenses are still a problem for CVB and members unanimously agreed on the importance of this funding in the 2011 final budget.