CONTROL ID: 2016969

TITLE: Enhanced Sensitivity of an Antibody Enzyme-linked Immunosorbent Assay using *Equine Arteritis Virus* Purified by Anion Exchange Membrane Chromatography

ABSTRACT BODY:

Narrative: In the present study, a rapid and easily-scalable method for purifying *Equine arteritis virus* (EAV) using an anion exchange membrane chromatography capsule (AEC) was developed. The relative advantage of AEC-purified EAV was evaluated based on the following parameters: 1) The presentation quality of the epitope defined by GP5-specific monoclonal antibody 17B7, and 2) The relative sensitivity of an antibody competitive enzyme-linked immunosorbent assay (cELISA) using AEC-purified antigen compared to an otherwise-identical commercial antibody cELISA using differential centrifugation-purified antigen. AEC-purified EAV antigen contained ~86.3% GP5 monomer while differential centrifugation-purified EAV contained less than 29.4% GP5 monomer. Improvement of cELISA analytical sensitivity without sacrifice of analytical specificity was clearly evident when cELISAs based on the two purification methods were evaluated using sensitivity check sets composed of borderline positive/negative sera from three horses vaccinated with a commercial modified live attenuated vaccine (MLV), and a time point serum set sequentially collected from an MLV-vaccinated horse. Furthermore, the AEC-purified antigen cELISA had 44.2% to 46.4% higher agreement with the virus neutralization (VN) test than the cELISA derived from differential centrifugation-purified EAV when tested with 43 borderline EAV-seropositive samples as defined by the VN test. In addition, the AEC-purified antigen cELISA had highly significant (p = 0.001) robustness indicated by intra-laboratory repeatability and inter-laboratory reproducibility when evaluated with the sensitivity check sets. The results suggest that the use of AEC-purified antigen in the cELISA may significantly contribute to further harmonization of the antibody cELISA with the OIE-prescribed VN test.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021246

TITLE: Evaluation of a Commercially Available Competitive ELISA (cELISA) for the Detection of Antibodies to *Equine Arteritis Virus* (EAV)

ABSTRACT BODY:

Narrative: *Equine arteritis virus* (EAV) is the causative agent of equine viral arteritis (EVA), an economically important respiratory and reproductive disease of horses. The virus neutralization test (VNT) is the principal serological assay used to detect evidence of EAV infection by most laboratories, and currently is the World Organization for Animal Health (OIE) prescribed test for EVA for international trade. The assay is used for diagnosis, epidemiological surveillance, trade, and pre and post vaccination screening of horses. Although the VNT is the most highly sensitive and specific serodiagnostic test for this infection, it has the disadvantage being expensive, labor-intensive, and time-consuming to perform. Furthermore, results can vary among laboratories when inadequate attention is paid to standardization of both test reagents and test protocol. Moreover, serum cytotoxicity caused by anti-cellular antibodies directed against RK-13 cells can present difficulties in test interpretation. To overcome these disadvantages, a commercial competitive enzyme-linked immunosorbent assay (cELISA) to detect antibodies to EAV has been developed. The

objective of this study was to compare the cELISA to the VNT for detection of EAV antibodies. A total of 3300 archived equine sera were tested by the commercially available cELISA and by VNT as the OIE approved gold standard. These included 768 sequential samples from horses experimentally inoculated with various EAV strains, 22 sera from horses inoculated with selected archived strains of EAV, and 1510 field samples randomly selected from those submitted for serological testing to the OIE EVA reference laboratory at the Maxwell H. Gluck Equine Research Center. The positive cELISA cut-off of $\geq$35% inhibition (%I) was used as recommended by the manufacturer. The cELISA results and VNT results from the experimental inoculated horses were used to calculate the specificity and sensitivity of the cELISA. In general, the pattern of antibody development determined by cELISA correlated very well with the VNT. However, seven samples positive by ELISA were negative by VNT (false positive) and four samples positive by VNT were negative by ELISA (false negative). The sensitivity and specificity of the cELISA were calculated to be 99.1% and 97.8%, respectively, compared to the VNT. These results confirm that the cELISA is highly specific and sensitive and when fully validated, may be accepted by the OIE as an alternative test to the VNT for detecting EAV-specific antibodies in equine sera.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021782

TITLE: Evaluation of a Newly Developed Insulated Isothermal RT-PCR Assay and a Real-Time RT-PCR Assay for the Detection of *Equine Arteritis Virus* Nucleic Acid in Equine Semen

ABSTRACT BODY:
Narrative: Prevention of venereal transmission of *equine arteritis virus* (EAV) is critical in minimizing the spread of the virus through the movement of carrier stallions or virus infective semen. Identification of the carrier stallion is therefore of critical epidemiological importance in the prevention and control of EAV infection. The objective of this study was to develop a reverse transcription insulated isothermal polymerase chain reaction (RT-iiPCR [POCKIT^{TM}]) targeting open reading frame 7 (*ORF7*) and compare it to a previously described real-time RT-PCR (rRT-PCR). The field-deployable POCKIT^{TM} system incorporates fluorescent probe-based iiPCR technology. It can provide test results from extracted nucleic acid within one hour. The limit of detection of RT-iiPCR was determined to be 10 copies of *in vitro* transcribed EAV *ORF7* RNA. Analysis of viral RNA extracted from 10-fold dilutions (10^0 to 10^{-10}) of tissue culture fluid containing the Virulent EAV Bucyrus strain (VBS) and the KY 84 strain showed that RT-iiPCR could detect EAV nucleic acid up to 10^{-6} and 10^{-7} dilutions, respectively, which was 10-fold higher than the rRT-PCR. Both assays generated positive signals with nucleic acid extracted from twenty-six known laboratory, field, and modified live virus vaccine strains. Both assays were highly specific and there was no cross reactivity with thirteen other important equine pathogens. Accuracy of both assays was evaluated against virus isolation (gold standard) for the detection of EAV in 118 archived sequential semen samples from experimentally infected carrier stallions. The relative sensitivity, specificity and accuracy were 98.41% (62/63), 100.00% (55/55), and 99.15%, respectively, for the rRT-PCR

(Cohen's kappa value 0.98), and 100.00% (63/63), 98.18% (54/55), and 99.15%, respectively for the RT-iiPCR (Cohen's kappa value 0.98). Finally, a spin column-based extraction method (PetNAD™ Nucleic Acid Co-prep kit) and a portable automatic extraction method (taco™ mini Automatic Nucleic Acid Extraction System) intended for point-of-need applications were shown capable of extracting EAV RNA comparable to the MagMAX™-96 Viral RNA Isolation Kit. The RNA extracted with taco™ mini had similar sensitivity to the other two extraction methods in the RT-iiPCR. These results suggest that both rRT-PCR and RT-iiPCR are equivalent for the detection of EAV in semen samples.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2018747

TITLE: Development of a Multiplex Assay to Determine Antibodies to Different Glycoproteins of *Equine herpesvirus 1*

ABSTRACT BODY:

Narrative: Development of rapid, sensitive and high-throughput assays for the assessment of immunity to *equine herpesvirus* type 1 (EHV-1) is important for vaccine development, clinical diagnostics and the evaluation of protective EHV-1 antibody levels in horses. The current gold standard is the serum neutralization (SN) test, which is time intensive and does not differentiate between EHV species. We have developed a new multiplex test based on two major EHV-1 antigens (glycoproteins C (gC) and gD). The antigens of interest were cloned and stably expressed in a mammalian expression system as IL-4 fusion proteins. Recombinant gC and gD antigens were then purified and coupled to fluorescent beads, which were used to quantify antigen-specific antibodies in horse serum on a Luminex platform. The assay was validated on serum samples from 58 horses. Validation samples included naturally EHV-1 infected, vaccinated or non-exposed horses with known SN titers ranging between <2 and 768. Multiplex-based antibody values were highly correlated with SN (R=0.87, p<0.0001). A group of 15 naïve horses were also monitored prior to and at monthly intervals following vaccination for one year to establish reference levels for protective immunity. In conclusion, the new multiplex EHV assay is an efficient alternative to SN testing.

CURRENT CATEGORY/DISCIPLINE: Virology | Epidemiology

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AWARDS:

Trainee Letter:

CONTROL ID: 2025725

TITLE: First Diagnosed Case of Vesicular Stomatitis in the United States in 2014

ABSTRACT BODY:

Narrative: *Vesicular stomatitis virus* (VSV) is a member of the *Rhabdoviridae* family mainly affecting horses, mules, donkeys, cattle, and swine. In the United States (US) the virus is usually seen in outbreaks in the southwestern part of the country. Typically oral vesicles, ulcers, and erosions are seen in and around the mouth, but animals can also manifest similar lesions around the feet, udder, or prepuce. There are 2 main serotypes seen in the US, New Jersey (VSV-NJ) and Indiana type 1 (VSV-IN1). The last outbreak of VSV-IN 1 occurred in 1998. The last outbreak of VSV-NJ was in the states of New Mexico and southern Colorado in 2012. On May 21, 2014 serum and swab samples from five horses located in Southern Texas were collected and submitted to the National Veterinary Services Laboratories (NVSL) for VSV Foreign Animal Disease (FAD) testing. Three of the five horses had crusty, swollen muzzles and vesicles of the oral mucosa on May 18. By May 21st two more horses had similar lesions while the lesions of the original three were resolving. NVSL tested the serum of all five by cELISA and compliment fixation (CF) tests and inoculated the swabs on cell culture for virus isolation (VI). Two of five horses had titers on the CF test to VSV-NJ. Three of the five horses were positive on the cELISA test for VSV-NJ. Two of the five horses (the two that were negative for cELISA and two of the three that were negative on the CF) were positive for VSV-NJ virus by isolation in less than 24 hours. Reisolation was performed to confirm the results. Whole genome sequencing indicated the virus was greater than 99% homologous to a strain from New Mexico associated with the 2012 outbreak.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021886

TITLE: SNP Analysis Used to Select Conserved Regions for an Improved *Newcastle Disease Virus* Real-time RT-PCR Test

ABSTRACT BODY:

Narrative: *Newcastle disease virus* is a RNA virus with high sequence diversity that may cause a severe disease in susceptible poultry. The severe form of the disease is considered a foreign animal disease in the United States and in many other countries, and rapid detection is critical for successful eradication when disease outbreaks occur. Real-time RT-PCR (rRT-PCR) has become the most commonly used test for screening clinical samples for the virus. Sequence mismatches of primers and probe to the circulating field strains has resulted in false negative tests or reduced sensitivity which can compromise our goal of rapid and early detection. With the rapid increase in the number of sequences of NDV available in public databases, it provides new opportunities for tests to be developed that are to the most highly conserved regions of the viral genome. A new approach for finding the best sites for primers and hydrolysis probes was developed using the single nucleotide polymorphism (SNP) analysis to calculate variability at every nucleotide of the genome and then use a boxcar average approach to identify the most conserved regions. A total of eight different regions that were highly conserved and were amenable to a rRT-PCR test were empirically tested to identify the most promising tests for additional study. Sensitivity, specificity, and end-point detection were considered after the most promising tests were optimized. Several promising tests were identified that could potentially replace or provide an alternative to the existing

matrix rRT-PCR test used in the U.S. The SNP analysis approach can be used for any pathogen for molecular diagnostic testing.

CURRENT CATEGORY/DISCIPLINE: Virology | Epidemiology
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AWARDS:
Trainee Letter:

CONTROL ID: 2016456

TITLE: Detection of H5 and H7 Highly Pathogenic Avian Influenza Virus with Lateral Flow Devices: Performance with Healthy, Sick and Dead Chickens

ABSTRACT BODY:
Narrative: Rapid detection of highly pathogenic avian influenza virus (HPAIV) in the field is critical for effective disease control and to differentiate it from other diseases, such as Newcastle disease. Lateral flow devices (LFD) are commercially available and provide a fast, highly specific, on-site test for type A influenza. Because of the low analytic sensitivity of LFD tests at low virus concentrations, targeted sampling of sick and dead birds has been proposed in order to increase detection probability. In order to quantify how clinical condition correlates to the detection of HPAIV with LFDs and whether delayed testing of dead chickens affects detection we exposed 50 chickens to a low dose of an H5 HPAIV and 50 chickens to a low dose of H7 HPAIV. Low doses were used in an attempt to increase mean death times. Oro-pharyngeal swabs were collected from all birds at 12, 24, 36, 48, 60, 72, 84, 96 and 108 hrs post exposure. During sample collection each chicken was scored as healthy, sick or dead. Half of the dead birds were placed in an empty isolator and samples were not collected until the next sample time, so swab collection was delayed 12hr. All swab samples were tested at the time of collection with a commercially available US licensed LFD for avian influenza virus and were subsequently tested with quantitative real-time RT-PCR to quantify the virus in each swab sample. With the combined data of both experiments 9.1% of healthy chickens, 82.4% of the sick chickens, 90.3% of the dead birds tested immediately, and 91.3% of the dead birds with 12hr delayed sampling were positive with the LFD. There was a direct correlation between the titer in the sample and whether the LFD was positive; the lowest titer the LFD could detect was $10^{4.6}$ 50% egg infectious doses per ml (EID50/ml) and at the titer of $10^{5.6}$ EID50/ml and above 100% of samples were positive. Delaying testing of dead birds by 12 hrs did not affect results and titers from swabs collected after a 12hr delay were significantly higher than those collected from freshly dead birds.

CURRENT CATEGORY/DISCIPLINE: Virology
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AWARDS:
Trainee Letter:

CONTROL ID: 2022750
TITLE: Characterization of H1N2 Variant Influenza Viruses in Pigs

ABSTRACT BODY:

Narrative: Introduction of the 2009 pandemic H1N1 virus (pH1N1) into swine herds has led to reassortment between the pH1N1 and endemic swine influenza viruses (SIVs) worldwide. Recently, reassortant H3N2 and H1N2 variants that contain only the M gene from pH1N1 and the remaining seven genes from North American triple-reassortant (TR) SIVs have emerged. These variant viruses have caused more than 300 cases of human infections and one death in the USA, creating a major public health concern. To date, the pathogenicity and transmissibility of H1N2 variant viruses has not been investigated using an animal model. Through passive surveillance of Kansas swine herds, we isolated 25 H1N2 SIVs: 16 of these viruses are reassortant viruses with genes from pH1N1 and 12 of those are variant viruses with only the M gene from pH1N1. This suggests that H1N2 variants with only M gene from pH1N1 have become established in Kansas swine herds. To further determine the pathogenicity and transmissibility of novel reassortant H1N2 viruses, we selected two reassortant H1N2 SIVs from our isolate pool to infect pigs: one is a swine H1N2 variant virus (swH1N2v) with the M gene from pH1N1; the other is a reassortant H1N2 virus (2+6 rH1N2) with two surface genes from endemic North American TR H1N2 SIVs and six internal genes from pH1N1, using a human H1N2 variant (huH1N2v) and an endemic TR H1N2 SIV (eH1N2) isolated in 2011 as controls. All four viruses were able to infect pigs and replicate in the lungs. Both H1N2 variant viruses caused more severe lung lesions in infected pigs when compared to the eH1N2 and 2+6 rH1N2 viruses. Although all four viruses are transmissible in pigs and were detected in the lungs of contact animals, the swH1N2v replicated more efficiently than the other three viruses in the respective sentinel animals. Additionally, the huH1N2v displayed delayed and inefficient nasal shedding in sentinel animals. Taken together, the swine and human H1N2 variant viruses are more pathogenic and the swH1N2v more transmissible in pigs and could pose a threat to public and animal health.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter: Michael Duff Role.pdf

CONTROL ID: 2020469

TITLE: Pathogenicity of Chimeric H17N10 Bat Influenza Virus in a Mouse Model

ABSTRACT BODY:

Narrative: The subtyping of *influenza A viruses* (IAV) is based on surface hemagglutinin (HA) and neuraminidase (NA) glycoproteins. Until 2010, 16 HA and 9 NA were known and all were found in the aquatic birds. The recent discovery of novel H17N10 and H18N11 bat influenza virus (BIV) genomes has challenged this traditional notion and expanded the natural host spectrum for influenza viruses. No live BIVs so far are available because they are not cultivable in substrates for known IAV which has been a major obstacle in further characterizing these viruses. In this study, chimeric BIVs (cH1N1/H17 and cH3N2/H17) containing HA and NA coding regions from known IAVs (PR8/H1N1 and TX98/H3N2) in the genetic background of H17N10 BIV were generated using reverse genetics. The chimeric BIVs replicated efficiently in Madin-Darby canine kidney (MDCK) cells comparable to the parental PR8/H1N1 and TX98/H3N2 viruses. To further investigate the pathogenicity of chimeric BIVs in vivo, 6-7 week old BALB/c mice were intranasally challenged with 103 TCID50 of both chimeric and parental viruses. The PR8/H1N1 and cH1N1/H17 caused 100% and 75% mortality respectively, whereas the TX98/H3N2 and cH3N2/H17 viruses were not virulent in mice. All the viruses replicated efficiently in mouse lungs. Histopathologically, both wild type and chimeric viruses were able to cause typical influenza like lesions characterized by varying degree of broncho-alveolar epithelial degeneration and necrosis and

interstitial pneumonia. Anti-influenza A nucleoprotein antibody showed moderate to strong immunoreactivity in all mouse lungs except cH3N2/H17 infected mouse which showed a rather weak immunoreaction. In summary, our experiments demonstrate that chimeric BIVs, which HAs and NAs from the IAVs but the packaging regions from the novel BIV replicate and are virulent in mammalian host. These chimeric viruses could be used as an invaluable tool to better understand the novel BIVs.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2027293

TITLE: Updating PCR Assays for Influenza Subtyping

ABSTRACT BODY:
Narrative: The recent event of a low pathogenic avian influenza A(H7N9) causing significant morbidity and mortality in humans from China prompted national veterinary laboratories worldwide to ensure the ability to detect this emergent strain. Diversity long recognized in the H7 lineage has resulted in the use of assays which target viruses circulating by geographic region, thus while the Type A assay detected the virus of concern, the regionally specific H7 subtype assay did not. Rapid evaluation of available molecular assays was made possible thanks to the timely sequence data shared by the Chinese Authorities to the public database at the Global Initiative on Sharing All Influenza Data (GISAID, www.gisaid.org), but complete validation of assays can be a long process, especially when needed for use by multiple labs. The current H5 assay has also been under revision to include detection of distinct viruses from Mexico identified as the result of an interlaboratory collaboration for the harmonization of IAV diagnostics between Mexico, Canada, and US. In these cases, it is important to review testing algorithms, identify and implement interim actions, and keep stakeholders informed of progress and next steps. The National Animal Health Laboratory Network (NAHLN) provides the framework needed for such communication and has served as a "proving" ground for harmonizing approaches to methods comparison and validation efforts. The approach to validation, progress to date, and lessons learned towards improved transparency and communication for the H5 and H7 assays will be reviewed.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2015594

TITLE: Characterization of a Novel Influenza Virus in Cattle with Bovine Respiratory Disease

ABSTRACT BODY:
Narrative: We previously reported the isolation of a novel virus, provisionally designated

C/swine/Oklahoma/1334/2011 (C/OK), with 50% overall homology to human *influenza C viruses* (ICV), from a pig in Oklahoma. The novelty of C/OK virus prompted us to investigate whether C/OK virus could exist in a non-swine species. Significantly, we found that the C/OK-like virus was widespread in U.S. bovine herds, as demonstrated by reverse transcription (RT)-PCR and serological assays. Genome sequencing of three bovine viruses isolated from two herds in different states further confirmed these findings and detailed studies on the biology of this virus suggested that it represents a new genus in the family *Orthomyxoviridae* (proposed influenza D virus, IDV). To further our understanding of the epidemiology of this virus in cattle, we screened 208 samples collected from cattle with bovine respiratory disease (BRD) that were submitted for diagnostic testing from multiple states. Approximately 5% of the samples were positive for IDV by RT-PCR. A similar percentage of samples (3.4-7.2%) were positive for established BRD pathogens *bovine viral diarrhea virus*, *bovine herpesvirus* 1 and *bovine respiratory syncytial virus* while *bovine coronavirus* was the most commonly identified virus (34% positive). Virus isolation for IDV was successful from 10 RT-PCR positive samples and full genome sequencing identified >96% identity to C/OK. Phylogenetic analysis of the hemagglutinin esterase protein identified two well supported clades. Hemagglutination inhibition assays using rabbit polyclonal antiserum generated against representatives from each clade further demonstrated significant (>4-fold) antigenic differences between viruses in the two clades. These results demonstrate that IDV is commonly present in animals with BRD and that genetic and antigenic variability is present in circulating viruses. Further work is necessary to investigate an etiological role for IDV in BRD. The finding of C/OK-like virus in swine and cattle suggests that this new virus may spread and establish infection in other mammals, including humans.

CURRENT CATEGORY/DISCIPLINE: Virology | Epidemiology

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AWARDS:

Trainee Letter: