CONTROL ID: 2022662

TITLE: Simultaneous Detection of *African Swine Fever Virus* Antibodies in Serum and Oral Fluid Using a Recombinant p30 Antibody ELISA

ABSTRACT BODY:
Narrative: African swine fever (ASF) is a devastating, highly contagious disease classified as a Foreign Animal Disease (FAD) in the U.S. Serology has been widely used in ASFV control programs in the Iberian Peninsula and Sardinia as a tool for the detection of ASFV carrier animals. Among ASFV proteins considered to be candidate antigens for serological tests, structural proteins p30, p54, and p72 are the best described, most highly studied, and most widely used in commercial ASFV serum antibody ELISAs1.
Serum and oral fluid antibody-positive samples were generated by experimental inoculation of 17 pigs with an attenuated ASFV isolate (NHV) that produces chronic infection. Oral fluid and serum samples were sequentially collected over days post inoculation (DPI 0, 6, 12, 15, 19, 26, 33, 40, 47, 54, and 61) using methods previously described2. The performance of the optimized ELISA was also evaluated using serum (n = 200) and oral fluid (n = 200) samples from animals (n = 400) known to be free of ASFV infection.
The antigen used in the ELISA was selected by evaluating the serum antibody response of ASFV-infected pigs against three recombinant antigens (rp30, rp54, rp72) using a multiplex fluorescent microbead-based immunoassay (FMIA; Luminex® Corporation). Antibody was detected at 6 DPI against p72 (11%) and at 12 DPI for both p30 (100%), and p54 (89%). All pigs (100%) were positive at DPI 12 for p30, at DPI 15 for p54, and at DPI 19 for p72. Recombinant p30 was selected as antigen target for subsequent development of an antibody ELISA.
ASFV rp30 antibody ELISA was able to detect ASFV antibodies by DPI 12 in both serum and oral fluid specimens run on the same plate simultaneously. The evaluation of known ASFV negative field samples showed specificities of 99.5% and 100% for serum and oral fluid samples, respectively. Given the increased surveillance efficiency provided by oral fluid sampling and the ability to corroborate results using serum samples, the ASFV rp30 antibody would be a highly useful under conditions that warrant ASFV surveillance.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2032126

TITLE: Development and Testing of a Multiplex Molecular Diagnostic Assay for Simultaneous Detection and Differentiation of Multiple Bacterial and Viral Causes of Respiratory Disease in Pigs

ABSTRACT BODY:
Narrative: A multiplex molecular assay for the rapid and sensitive diagnosis of respiratory disease in pigs has been developed. The assay enables detection and differentiation of 4 bacterial (*M. hyponeumoniae, A.pleuropneumoniae, H.parasuis,* and *S.suis*) and 6 viral pathogens (PRRS, Flu, PCV2, Pseudorabies Virus, ASF, CSF) with no cross-reactivity to

clinical and genetic near-neighbor organisms. In addition to endemic diseases, the assay is able to detect and differentiate between African Swine Fever and Classical Swine Fever. The 25-plex assay has sensitivity of 1000 infectious units or less per mL of porcine oral fluid. For Influenza detection, an LOD of 200 infectious units per mL has been demonstrated. The assay is expected to be of utility in clinical diagnosis of important endemic diseases as well as surveillance for FADs.

CURRENT CATEGORY/DISCIPLINE: Virology | Bacteriology/Mycology

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AWARDS:

Trainee Letter:

CONTROL ID: 2010394

TITLE: *African Swine Fever Virus*, *Classical Swine Fever Virus*, and *Foot-and-Mouth Disease Virus* Detection By Multiplex Reverse Transcription Quantitative Polymerase Chain Reaction in Swine Oral Fluids

ABSTRACT BODY:
Narrative: African wine fever (ASF), Classical swine fever (CSF), and Foot-and-mouth disease (FMD) are highly contagious animal diseases of significant economic and social importance. Pigs infected with ASFV and CSFV develop clinical signs that may be indistinguishable from other diseases. Likewise various causes of vesicular disease can mimic clinical signs of FMD. Early detection is critical to limiting the impact and spread of these disease outbreaks, and the ability to perform surveillance for all three diseases rapidly and cost effectively using a single diagnostic sample is highly desirable. This study assessed the feasibility of simultaneous ASFV, CSFV, and FMDV detection by multiplex reverse-transcriptase quantitative polymerase chain reaction (mp RT-qPCR) in swine oral fluids simply collected through use of a chewing rope. Animal groups were experimentally infected independently with each virus, observed for clinical signs, and oral fluids collected and tested throughout the course of infection, in four independent studies conducted months apart. All animal groups chewed on the ropes readily before and after onset of clinical signs and before the onset of lameness or serious clinical signs. ASFV was detected as early as 3 days post infection (dpi), 2-3 days before onset of clinical disease; CSFV was detected at 5 dpi, coincident with onset of clinical disease; and FMDV was detected as early as 1 dpi, 1 day before the onset of clinical disease. Equivalent results were observed in four independent studies and demonstrate feasibility of oral fluids and mp RT-qPCR for surveillance of ASFV, CSFV, and FMDV in swine populations.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2031153

TITLE: Early Post Natal CSFV Infection Can Result in Persistently Infected Piglets

ABSTRACT BODY:

Narrative: Classical swine fever (CSF) remains one of the leading threats to the pig industry worldwide. In recent years, CSFV has been prevalent essentially in Asia and in Central and South America, with sporadic occurrence in Europe also. The broad range of disease severity observed with CSFV infections in pigs depends on the virulence of the strain and on the age, breed, health and immune status of the pig. Interestingly, a trend towards milder, chronic clinical manifestations of CSF has been observed particularly in endemic countries such as Cuba and China. Pigs infected with low-virulent strains appear mostly healthy and shed virus continuously or intermittently for months, representing a major problem for disease control. The pathogenesis of such unapparent infections and their implications for virus progression in pig populations especially in endemic regions is poorly understood. The presence of low virulent CSFV strains in the field has mainly been related to the "pregnant carrier sow syndrome" involving trans-placental infection of the foetus leading to persistently infected piglets. In contrary, the role of post natal infection for viral persistence in the field is poorly documented and not well understood. Therefore, the aim of this study was to evaluate the ability of CSFV to induce viral persistence upon early postnatal infection. This was explored with two recent low virulent CSFV field isolates, an endemic virus from Cuba and a recent isolate from Spain. Two litters of 10 piglets were infected via the intranasal route at the day of birth with the Cuban and the Spanish isolate, respectively. During the observation period of 6 weeks post infection, most of the infected piglets remained clinically healthy despite persistent high virus titres in the serum. Importantly, these animals were unable to mount any detectable humoral immune response. No virus could be isolated from serum of the two sows and of the few piglets that had seroconverted. This study shows that early post natal infection can result in a high proportion of seronegative persistently infected piglets, which has important implications for disease control and eradication in countries where CSFV is currently endemic.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2020404

TITLE: Genetic Variation Observed in BVDV Isolated from 34 Persistently Infected Cattle Generated in One Outbreak

ABSTRACT BODY:
Narrative: *Bovine viral diarrhea viruses* (BVDV) are single stranded RNA viruses that belong to two different species within the pestivirus genus of the Flavivirus family. Similar to other viruses with single stranded RNA genomes, BVDV isolates exist as quasispecies, or a swarm of individual viruses, each with small numbers of unique nucleotide differences. Exposure to bovine viral diarrhea viruses may result in acute and persistent infections. Persistent infections are the result of exposure in utero prior to 125 days gestation. Persistently infected (PI) cattle are immunotolerant to the viral strain with which they were infected in utero which prevents them from clearing the virus and results in viral replication in numerous tissues and lifelong shedding of the virus. Previously it has been shown that a greater number of nucleotide substitutions were introduced into the BVDV viral genome during acute infections of pregnant cattle than of non-pregnant cattle. In this study we looked at the stability of the BVDV genome from viruses isolated over time in 34 PI cattle generated during one outbreak of BVDV2. The outbreak occurred in a group of 136 bred heifers assembled by a cattle buyer and purchased in 2004 to increase a research herd. Between March and May of 2004, 128 live calves were born of which 8 died as neonates. A total of 41 out of the remaining 120 calves tested positive for persistent BVDV infection based on positive tests on 2 samples collected at least two weeks apart. Of this group, 34 survived long enough to have blood samples collected in December of 2004 and February and March of 2005. Virus was isolated from the buffy coat of each animal at each sampling point and the 5' UTR and E2 region sequences generated by PCR amplification followed by cycle sequencing. Phylogenetic analysis revealed that the viruses were highly similar but belonged to two distinct clades. The viruses isolated at the three different time point from 14 of the 34 cattle were identical in even the highly variable region of the E2. Minor differences were observed among viruses isolated from the other cattle at different time points. Segregation into the two clades was consistent for viruses across all the time points. To determine if viral variants from both clades were part of the viral swarm for each of the isolates sequencing of individual clones was performed. This was done by cloning the E2 PCR amplicon generated from the March buffy coat samples into a bacterial vector and then sequencing at least 100 of the recombinant vectors. Sequencing revealed that the viral swarms from isolates segregated to one clade did not include variants that matched the other clade. These results suggest that while variants are generated at a higher rate following infection of pregnant animals, infection of the fetus resulting in persistent infection is the result of infection with a single variant.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2016859

TITLE: One-Step Triplex Real Time RT-PCR Assay for Simultaneous Detection and Differentiation of Three Vesicular Viruses in Swine

ABSTRACT BODY:
Narrative: Vesicular Stomatitis (VS) and Swine Vesicular Disease (SVD) are two worldwide livestock diseases that are of economical importance. They cause vesicular lesions, ulcerations of the tongue and oral tissues, and coronary bands in infected animals. VS has two major serotypes that are *Vesicular stomatitis Indiana virus* (VSIV) and *Vesicular stomatitis New*

Jersey virus (VSNJV). SVD only has one serotype, but animals are often co-infected with VS strains, making it difficult for accurate clinical diagnosis. Therefore, rapid detection and accurate differentiation of these viruses is critical for effective disease management. Here we describe a novel one step triplex real time RT-PCR for simultaneous detection and differentiation of VSIV, VSNJV and SVDV. The most reserved region of the L gene of VSV and the 5'UTR of SVDV were selected as detection targets. Our results showed that the multiplex assay generated similar sensitivity levels as compared to its corresponding single-target PCRs. The amplification efficiencies of multiplex real time PCRs were 96.1%, 98.6% and 98.1%, and the correlation coefficients of Ct values from the standard curves generated by the multiplex reaction and its corresponding singular reactions were 0.9994, 0.9953 and 0.9995, for VSIV, VSNJV and SVDV, respectively. The detection limits of triplex real time PCRs were about 10 copies per reaction for the three viruses, which are comparable to its corresponding singular real time PCR reactions. When primers and probes of the three viruses were used in the same reaction on individual virus template, only the corresponding channel generated signal, and there is no cross-reaction or interference observed. Similar specificity was also obtained from singular reactions. This assay may be a cost-effective alternative for rapid and accurate detection and differentiation of VSIV, VSNJ and SVDV strains. This assay may be especially useful when co-infections with two or three of these viruses occur in the same animal.

CURRENT CATEGORY/DISCIPLINE: Virology | Bacteriology/Mycology

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AWARDS:

Trainee Letter:

CONTROL ID: 2032878

TITLE: Establishing Critical Diagnostic Capability for Foot-And-Mouth Disease in Red Deer

ABSTRACT BODY:

Narrative: New Zealand has the largest population of farmed red deer in the world (1.1 million) along with a significant feral deer population. Deer are susceptible to foot-and-mouth disease (FMD) although clinical signs for FMD in deer are much less severe than in other ruminants. Consequently, during any FMD outbreak in New Zealand laboratory testing of large numbers of red deer samples would potentially be required. Currently the accuracy of available diagnostic tests for detecting FMD in red deer is unknown as these tests have been developed for other livestock species. The aim of this project was to determine which diagnostic tests are the most appropriate for use with red deer. This was a collaborative research project between the National Centre for Foreign Animal Disease (NCFAD), Winnipeg, Canada and the Animal Health Laboratory (AHL), Ministry for Primary Industries in New Zealand.
At NCFAD 10 red deer were intra-nasally inoculated with the FMD serotype O virus, monitored for clinical signs, and samples taken regularly over a four week period. Samples were tested by RT-PCR, virus isolation, antigen detection using pen-side tests and ELISAs and antibody detection by the virus neutralization test (VNT), two O-serotype specific structural protein (SPO) ELISAs, and four serotype-independent non-structural protein (NSP) ELISAs and a NSP pen-side test.
Only one animal developed clinical signs. It tested positive by RT-PCR in various swabs, lesion materials and serum. In an in-house NSP-ELISA, a commercial NSP-ELISA and an antibody NSP pen-side test, the same animal showed positive results from day post inoculation (dpi) 14 onwards. Two other commercial NSP ELISAs detected anti-NSP serum

antibodies with lower sensitivity. The animal became positive in the VNT and an in-house SPO-ELISA at dpi 9 and in a commercial SPO-ELISA on dpi 11. Another three animals were RT-PCR positive only in nasal swabs.
Six of the red deer that were RT-PCR negative were re-inoculated intramuscularly with the same O-serotype FMDV at dpi 14 after the first inoculation. None of these animals became RT-PCR or NSP-ELISA positive but all six animals became positive in the VNT, the in-house SPO-ELISA and the commercial SPO-ELISA.
Currently at the AHL in New Zealand further evaluation of diagnostic specificity and sensitivity is being undertaken on the diagnostic tests that performed well at NCFAD using inactivated samples collected from the experimentally infected red deer and samples from FMDV free red deer from New Zealand.
In summary, this study has provided evidence that red deer are not easily infected with the FMD serotype O virus. The experimental infection of 10 red deer has shown that RT-PCR, a commercial NSP-ELISA, SPO-ELISA and a NSP pen-side test demonstrate good specificity and sensitivity for detecting FMDV infection in red deer. On-going work is further evaluating the performance of these tests in New Zealand.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2022156

TITLE: Detection of BVDV in Cattle Semen- How Common are Persistent Testicular Infections?

ABSTRACT BODY:
Narrative: The detection of BVDV in the semen of bulls is well known. Persistently infected (PI) bulls shed virus continuously and at a high titer and will result in the infection of all susceptible females that are inseminated. While not presenting as high a risk, semen from acutely infected bulls may also contain BVDV. Virus may be detected for a short time at and soon after the period in which the bull is viremic. Virus levels tend to be low but can occasionally infect a susceptible female, presenting a risk for the introduction of BVDV into a naïve herd or into a BVDV free population. Until recently, persistent testicular infections (PTI) were considered to be extremely rare. Prior to the last decade, only one case had been reported globally but in the last 3-4 years, several cases have been detected in Australia and the USA.
Virus levels in the semen of bulls with PTIs can be variable but at times high and could present a significant threat to susceptible herds. Further, it is expected that semen from a bull infected with a BVDV2 virus would initiate infection in a herd where there is only immunity to BVDV1 (either naturally or by vaccination). Detection of PI bulls is straightforward and PI bulls are now unlikely to reach a semen collection center. Even if the serological status of a bull is not known prior to arrival at a collection center, it is usual that there will be a 3-4 period of isolation, during which semen is not eligible for dispersal. During this time, it is likely that an acute infection will have resolved. However, PTIs are more problematic. To ensure that an animal does not have a PTI, the semen of all seropositive animals must be screened. As a result of intermittent virus detection in semen, several collections must be tested over several weeks to ensure that a bull is free of BVDV.
Virus isolation in cell culture has been the most common method to screen semen for freedom from BVDV. However, there are several disadvantages including toxicity of the semen,

presence of bacteria, the need to culture relatively large quantities of valuable semen for optimal sensitivity and a comparatively long time to obtain results, with associated high cost. There can also be issues with differences in performance of virus isolation between laboratories. Transport of samples to the laboratory must use a reliable cold chain, ideally using LN, to avoid reduction in virus infectivity during transport or handling in the laboratory. Real time PCR offers many advantages for the screening of semen, including rapid turn around, lower cost, and high analytical sensitivity that is rarely compromised by suboptimal transport or storage. This presentation will compare virus isolation and real time PCR for BVDV using a standard magnetic bead based nucleic acid extraction on semen from bulls with PTI. A pan-pestivirus assay is recommended. In all situations, real time PCR gives results that are superior to and more consistent than those obtained by virus isolation.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2022133

TITLE: Evaluation of Real-Time PCR Assays for the Detection of Viruses in Semen of Livestock

ABSTRACT BODY:

Narrative: Artificial breeding is widely used with livestock for the efficient transport and use of breeding animals with superior genetic characteristics. Semen and embryos are transported both nationally and internationally. While embryos can be washed and treated to minimize the likelihood of transfer of viruses, there are greater challenges with the supply of semen that is free of viruses of concern. While donor animals can be screened and be shown to be free of infection with some pathogens, there are many situations where this is not possible. There are many diseases where semen is collected from seropositive animals and although the donor is no longer viremic, virus may be present in the semen. Consequently, health protocols for semen collection centers often require semen to be screened for freedom from viral contamination. Examples included BVDV, BHV1, BTV & EHDV in cattle, EAV in horses and PCV2 and PRRS in swine. For some countries, other agents such as *Akabane* and *Schmallenberg viruses* can be a concern in cattle semen and *Bungowannah virus* from pig semen.

Virus isolation in cell culture has been the most frequently used technique to screen semen for freedom from viral infections. However, there are several disadvantages with the use of cell cultures. These can include toxicity of the semen to the cells, presence of contaminating bacteria, the need to culture relatively large quantities of valuable semen for optimal sensitivity and a comparatively long time to obtain results, with associated high cost. There can also be issues with differences in performance of virus isolation between laboratories. Finally, transport of samples to the laboratory must use a reliable cold chain, ideally using liquid nitrogen, to avoid reduction in virus infectivity during transport or handling in the laboratory.

With the availability of real time PCR protocols (qPCR or qRT-PCR for RNA viruses) for most commonly encountered viral infections, this technology offers many advantages over virus isolation for the screening of semen. These include rapid turn around, lower cost, and very high analytical sensitivity that is rarely compromised by suboptimal transport or storage conditions. This presentation will provide comparisons of virus isolation and real time PCR for a wide range of viruses using a standard magnetic bead based nucleic acid extraction protocol and common PCR reaction conditions for all viruses. Only the primers and probes vary for each individual virus. In all situations, real time PCR gives results that are superior to

and more consistent than those obtained by virus isolation in cell culture and should be recommended as a preferred method for the screening of semen for freedom from virus contamination.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2014291

TITLE: Rapid and Sensitive Detection of *Canine Distemper Virus* by One-Tube Reverse Transcription-Insulated Isothermal Polymerase Chain Reaction

ABSTRACT BODY:

Narrative: *Canine distemper virus* (CDV) has been associated with outbreaks of canine infectious respiratory disease in shelters and boarding kennel environments. Aiming to aid point-of-care diagnosis of canine distemper, a simple CDV detection method was developed on the basis of hydrolysis probe-based insulated isothermal polymerase chain reaction and POCKIT™ Nucleic Acid Analyzer. Analytical sensitivity (limit of detection) of the established CDV reverse transcription (RT)-iiPCR was about 11 copies of in vitro transcribed RNA per reaction. CDV RT-iiPCR generated positive signals from CDV, but not *Bordetella bronchiseptica*, *canine parvovirus*, *canine herpesvirus*, *canine adenovirus*, *canine influenza virus* (subtype H3N8), *canine parainfluenza virus*, and *canine respiratory coronavirus*. To evaluate accuracy of the established reaction in CD clinical diagnosis, 110 specimens from dogs suspected with CDV infection were tested simultaneously by CDV RT-iiPCR and real-time RT-PCR. CDV RT-iiPCR demonstrated excellent sensitivity (100%) and specificity (100%), compared to real-time RT-PCR. Generating reliable results from clinical samples within an hour using a portable device, the established method has great potential to be used for point-of-care diagnosis of CD.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2022751

TITLE: The Effect of Coagulant Treatment on the Constituents of Swine Oral Fluid Specimens

ABSTRACT BODY:

Narrative: Swine oral fluid specimens are commonly used for monitoring bacterial and viral infections in commercial swine populations using either PCR- or antibody-based assays. The focus of the work presented herein is improvement in assay performance by "cleaning up" the sample itself. Coagulants are commercially available, inexpensive chemical agents used in wastewater cleanup. The aim of this study was to determine the effect of a variety of

coagulants on the innate properties of porcine oral fluids.
Oral fluid samples submitted to the ISU-VDL for routine testing were aggregated to a total volume of 5.75 L, stirred to assure uniformity, and aliquotted into 30 ml volumes. Nine commercially available coagulants were evaluated against a negative control at 9 concentration levels (0.01, 0.1, 1, 10, 100, 250, 500, 750, 1000 ppm) for their effects on pH (UB-5, Denver Instrument, Inc.), mineral content, total protein, and turbidity (2100 AN, Hach Industries). To perform the testing, all samples were stored at 4°C until treated with the coagulant, after which they were agitated, centrifuged, and the liquid fraction was immediately separated and analyzed. Total protein concentration was determined using a BCA protein assay (Pierce™), and the trace content of magnesium, phosphorous, potassium, calcium, manganese, iron, copper, zinc, selenium, and molybdenum was measured using inductively coupled plasma spectrometry (820, Varian Inc.).
In general, as the concentration of coagulant increased, pH also increased between 6.5 and 6.8, although not when the coagulant contained a metal cation. The log nephelometric turbidity units (NTU) consistently decreased as the coagulant concentration increased, while total protein concentration was not affected (i.e., decreased) by any of the 9 nine coagulants except one. Coagulant treatment consistently and significantly decreased iron and copper concentrations; whereas zinc and phosphorous were variably affected, depending on the coagulant.
The fact that coagulants achieve a marked decrease in suspended solids (i.e., decreased turbidity) with minimal effect on total protein and pH suggests that coagulant treatment could improve the handleability and diagnostic utility of swine oral fluids.

CURRENT CATEGORY/DISCIPLINE: Virology

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