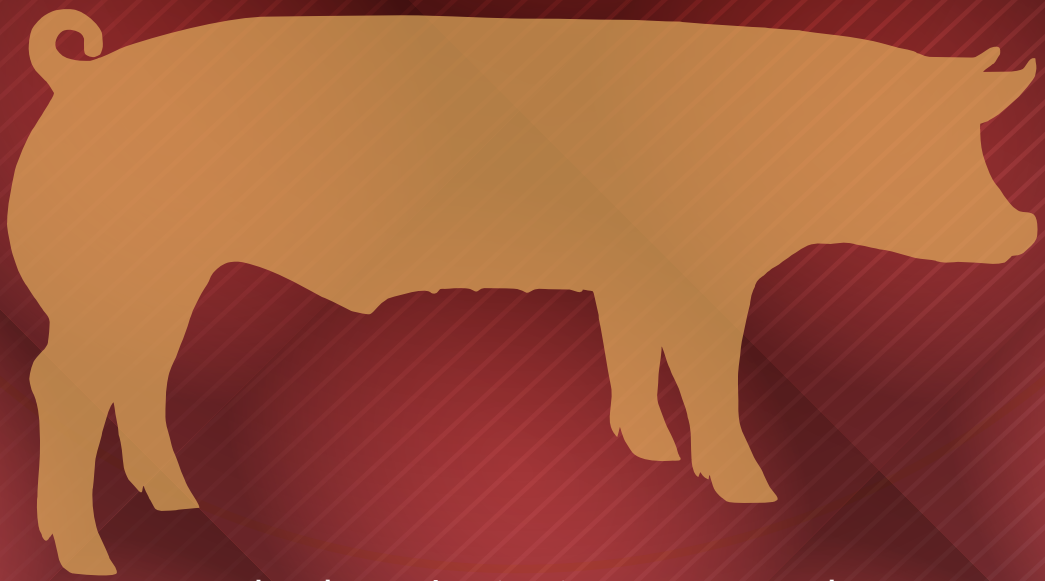
2009-2018

FOREIGN ANIMAL DISEASE RESEARCH

This book provides producers, veterinarians and veterinary researchers an informational resource of Checkoff-funded research that focuses on foreign animal diseases. For more Checkoff-funded swine disease research, visit *pork.org/research*.



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Introduction

Many herd-health challenges exist for today's pork producers. Diseases that are foreign to the United States, called foreign animal diseases (FADs), present a significant threat. According to a Checkoff-funded study, prevention of foot-and-mouth disease (FMD) is estimated to be worth $137 million per year to the U.S. pork industry[1]. Freedom from diseases such as Classical Swine Fever (CSF), African Swine Fever (ASF) and FMD provide more than a $55-per-head value to U.S. pork producers. Understanding the mechanisms of infection, transmission and maintenance in both the environment and pork products help the pork industry prepare to combat FADs through disease prevention, rapid detection and vaccine and biosecurity countermeasures.

The National Pork Board's Swine Health Committee understands the need to devote Checkoff resources for swine-specific research on FADs. Since 2004, the Checkoff 's calls for research proposals have resulted in more than 33 projects totaling more than $2.8 million. The Checkoff investment in swine disease research has helped support scientists in more than 25 universities, in many USDA laboratories and private facilities in the United States and abroad.

The National Pork Board has worked with groups and industry partners, including the American Association of Swine Veterinarians, the USDA and the Center of Excellence for Emerging and Zoonotic Animal Diseases at Kansas State University to leverage Pork Checkoff funds for swine health research and to foster collaboration and coordination of research priorities. The priorities for Checkoff research are developed in cooperation with, and complement USDA priorities. The goal is to provide swine herd health solutions to producers and their veterinarians for devastating and costly diseases. Criteria for research activities are outlined by the National Pork Board Strategic Plan, which include the following: 1. High impact to producers; 2. No overlap with other activities; and 3. Industry-wide benefit. The FAD research focus has included the areas of immunology, pathology, epidemiology, disease transmission, diagnostic testing and vaccine development and evaluation.

Additional Resources

Additional resources on swine disease research can be found at:

- Center for Food Security and Public Health *www.cfsph.iastate.edu*
- Swine Health Information Center *www.swinehealth.org*
- USDA Agricultural Research Service *www.ars.usda.gov/research*
- USDA National Institute of Food and Agriculture Research Reports: *www.nifa.usda.gov/tool/cris*

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[1] Economy Wide Impacts of a Foreign Animal Disease in the United States; Working Paper 11-WP 525, November 2011; Center for Agricultural and Rural Development, Iowa State University. 50011-1070

[2] www.card.iastate.edu.

Foot and Mouth Disease Virus (FMDV)

(11-005) Rational design of attenuated foot-and-mouth disease virus strains for development of improved disease countermeasures.

FMD is the one of the most feared viral disease that can affect livestock, including swine. Although this disease appeared to be eliminated from most developed nations by the end of last century, recent outbreaks in Europe, Japan, Taiwan, South Korea, Eastern Russia, etc, have demonstrated that infection can spread as wild fire affecting any nation and causing devastating economic and social consequences. Furthermore, post 9/11 the US is threatened by the potential deliberate release of FMDV by terrorist groups. Therefore, it is essential to develop new control strategies that could confer very early protection and stop disease spread. It has been demonstrated for other viral diseases that live-attenuated vaccines are one of the best choices to obtain a strong early and long-lasting protection. The current FMD vaccine is a formulation of inactivated WT virus antigen prepared in high containment bio-security level 3 facilities. This vaccine requires 7 days to induce protection, a time during which vaccinated animals are still susceptible to one of the fastest replicating viruses in nature. In addition the vaccine is prepared from highly virulent circulating virus strains that despite extreme caution and care in the manufacturing process, could result in outbreaks by accidental virus release as it happened in the United Kingdom in 2007 (estimated cost $2B). Our goal is to develop alternative control strategies that could improve current FMD countermeasure programs. An attenuated vaccine is expected to elicit more rapid innate immunity and a long lived adaptive immunity to effectively control disease. Moreover, induction of innate immunity could result in early protection against multiple FMDV serotypes. From the production perspective, use of an attenuated FMDV strain will reduce the consequences of accidental outbreaks caused by accidental release of virus from vaccine manufacturing facilities. Importantly, attenuated strains are excellent new tools to study the interactions between FMDV and the host immune system and ultimately could lead to the development of novel strategies to counteract FMD. During the past year and with the support of NPB we have successfully derived a mutant strain of FMDV that did not cause disease in swine (FMDV-SAP mutant). Interestingly inoculation of swine with this mutant strain induced a strong immune response that protected animals against infection with the parental (wild type) virus, as early as two days post vaccination. Studies in animals and in cultured swine cells demonstrated that, in contrast to the parental wild type virus, the mutant variant was unable to block some inflammatory responses thus limiting dissemination of the virus beyond the original site of inoculation. Furthermore, we have started studies to add more mutations to this virus aiming to increase the stability of the original mutations therefore decreasing the probability of reversion to virulence. Our results indicated that manipulation of the viral genome in the region that encodes for the leader protein is a viable alternative to derive less pathogenic FMDV strains that could be used as the basis for live attenuated vaccines against FMD or as seeds to grow the virus for manufacturing safer inactivated vaccines. Although this strategy is far from being used in countries that are FMD free without vaccination, it could be an affordable alternative to control FMD in regions of the world where the disease is enzootic thereby decreasing the risks of dissemination to disease-free nations. Ultimately a combination of strategies tailored to each region of the world will eventually succeed to eradicate this feared disease.

(11-174) Investigating potential existence of chronic, persistent foot-and-mouth disease virus infection in domestic pigs; implications for disease control strategies

The severe measures routinely enacted for control of foot-and-mouth disease (FMD) outbreaks are based upon extreme contagiousness of the disease, rapidity of spread, and fear of residual contagion associated with long-term asymptomatic virus carriers. It is well established that many ruminant species are capable of this prolonged carrier state; however, it is largely believed that pigs are incapable of becoming FMD virus (FMDV) carriers. The purpose of the work described herein was to investigate the potential for pigs to become long-term carriers of FMDV with the ultimate goal of using this information to design rational approaches to species-specific outbreak response planning. A large proportion of FMD research has focused on pathogenesis and prevention in cattle. The work performed within this project contributes detailed knowledge of the early and late stages of FMDV infection in pigs, which is critical to development of improved FMDV countermeasures for use in pigs. The FMDV carrier state in ruminants (cattle, sheep, etc) is a period subsequent to clinical disease in which animals carry and shed infectious FMDV for long periods without showing any symptoms of the disease. Because of this, an animal that has recovered from the disease, or that has been protected from developing disease by vaccination, is treated as if it may still be capable of spreading the virus. This means that large numbers of healthy animals are routinely destroyed during FMD outbreaks in countries that are normally free of the disease (USA, UK, France, etc). Based on limited data, it has become generally accepted as 'conventional wisdom' that pigs are not capable of becoming long term carriers of FMDV. However, during recent years, there have been a limited number of scientific publications that have suggested otherwise. The main purpose of the work performed within this project has been to thoroughly investigate if pigs are capable of harboring infectious FMDV for extended time after recovery from the disease.

To achieve these goals, pigs were experimentally inoculated with one of five distinct strains of FMDV. Infection dynamics were characterized for periods ranging from 6 hours to 100 days after which pigs were euthanized for tissue collection. These investigations demonstrated that infectious virus could not be detected in blood or secretions from pigs once the clinical signs of disease had disappeared. Furthermore, live virus was not detected in tissue samples obtained at 35-100 days after infection from pigs that had recovered from the disease. Although live virus was not recovered, viral degradation products (RNA and structural proteins) could be detected in select lymph nodes harvested at 35 days after infection. This detection of viral remnants declined markedly by 60 days after infection, and was completely absent at 100 days post infection. The data described herein provides the most extensive investigation of potential FMDV persistence in pigs. The overall conclusion is that domestic pigs are unlikely to carry infectious FMDV for prolonged periods after recovery from clinical disease. The significance of detection of viral degradation products in lymph nodes is debatable and has not been thoroughly investigated in the current studies. Overall, this work provides a basis for considering species-specific control plans. However, further research and validation would be required to enact such policy.

(12-023) Exploiting the potential of leader proteinase coding sequence of foot-and-mouth disease virus to derive attenuated strains suitable for pathogenesis studies and development of improved countermeasures

FMD is the one of the most feared viral diseases that can affect swine. Although this disease appeared to be eliminated from most developed nations by the end of last century, recent outbreaks in Europe, Japan, Taiwan, South Korea, Eastern Russia, etc, have demonstrated that infection can spread as wild fire affecting any nation and causing devastating economic and social consequences (Figure 1). Furthermore, post 9/11, a new era has emerged showing that any country is vulnerable to national and international terrorism. An outbreak of this disease would be devastating to the US economy which deals with the largest livestock market of the world. Therefore, it is essential to develop new control strategies that could confer very early protection and stop disease spread. FMD is a highly contagious disease that spreads very quickly among susceptible animals. The current FMD vaccine is a formulation of inactivated wild type (WT) virus antigen prepared in high containment bio-security level 3 facilities. This vaccine requires 7 days to induce protection, a time during which vaccinated animals are still susceptible to disease mostly when exposed to

FMD virus (FMDV). In addition the vaccine is prepared from highly virulent circulating virus strains that despite extreme caution and care in the manufacturing process, could result in outbreaks by accidental virus release as it happened in the United Kingdom in 2007 (estimated cost $2B). It has been demonstrated for other viral diseases that live-attenuated vaccines are one of the best choices to obtain a strong early and long-lasting protection. Our goal is to develop alternative control strategies that could improve current FMD countermeasure programs based on the development of attenuated vaccines. An attenuated vaccine is expected to elicit more rapid innate immunity and a long lived adaptive immunity to effectively control disease. Moreover, induction of innate immunity could result in early protection against multiple FMDV serotypes. From the production perspective, use of an attenuated FMDV strain will reduce the consequences of accidental outbreaks caused by accidental release of virus from vaccine manufacturing facilities. Importantly, attenuated strains are excellent new tools to study the interactions between FMDV and the host immune system and ultimately could lead to the development of novel strategies to counteract FMD. In the last couple of years with the support of NPB we have successfully derived a mutant strain of FMDV that did not cause disease in swine (FMDV-SAP mutant). Interestingly inoculation of swine with this mutant strain induced a strong immune response that protected animals against infection with the parental WT virus, as early as two days post vaccination. However in rare occasions, revertants with increased virulence spontaneously arose, indicating that improvement is required for safety. Utilizing reverse genetic approaches we had explored the possibility of adding more mutations in the SAP mutant to increase stability and attenuation. We had observed that some mutations in other Leader domains, like FHA domain, (e. g. H*) cause attenuation independently of SAP, however double mutants (SAP, H*) did not produce viable virus. In contrast, other SAP-derived strains with mutations in another domain of the L protein (CTE) are viable. We are currently characterizing the SAP-CTE mutants for further development into a live attenuated vaccine platform. Our results indicated that manipulation of the viral genome in the region that encodes for the leader protein is a viable alternative to derive less pathogenic FMDV strains that could be used as the basis for live attenuated vaccines against FMD or as seeds to grow the virus for manufacturing safer inactivated vaccines. Although this strategy is far from being used in countries that are FMD free without vaccination, it could be an affordable alternative to control FMD in regions of the world where the disease is enzootic thereby decreasing the risks of dissemination to disease-free nations. Ultimately a combination of strategies tailored to each region of the world will eventually succeed to eradicate this feared disease.

(14-014) Improvement of interferon bio-therapeutics for Foot-and-mouth disease in swine

Vaccination is one of the best strategies to prevent viral diseases; however, it usually takes 1 - 2 weeks for vaccines to induce protective immunity. Animals may become sick if they come into contact with the pathogens before the establishment of protective immunity. Foot-and-mouth disease virus (FMDV) is one of the most contagious animal viruses that could have devastating effects on livestock industries. There are commercial FMD vaccines available. The elapsed time of full protection for FMD vaccines is about one week. Development of a countermeasure with rapid onset of immunity will greatly facilitate the control of this disease. Biotherapeutics using a replication-defective recombinant adenovirus carrying an interferon gene can fully protect pigs from FMDV infection 24 hours after treatment. However, the disadvantages of this biotherapeutics are the requirement of high dose and short-lasting effect. The objective of this research is to enhance the potency of this adenovirus-based interferon biotherapeutics to overcome these drawbacks. We applied several strategies to produce three new recombinant adenoviruses. All these recombinant viruses induced higher anti-FMDV activity in cell culture than the adenovirus previously tested. We then selected the top two new and the previously tested adenoviruses to test in pigs. Based on the antiviral activities induced in the sera of treated pigs, the best adenovirus is greater than 20 fold more potent than the previous recombinant virus. It induced not only significantly higher but also longer lasting anti-FMDV activity even at a dose ten times lower than the previous virus. Our future plans are to determine the protective dose of this new biotherapeutics against viral challenges in pigs and to develop a system to produce this recombinant virus efficiently. There are still approaches that can be used to improve this biotherapeutics further and eventually make it feasible for commercial production.

(14-030) Use of animal movement data and epidemiological modeling to identify premises at high risk of infection in the event of a foot-and-mouth disease epidemic

Foot and mouth disease (FMD) is one of the most contagious and economically relevant diseases affecting livestock and is rapidly transmitted between hosts. Fortunately, the US swine industry has not experienced an FMD epidemic for almost a century. However, the recent introduction of pathogens, such as PED, demonstrate the need for increasing the industry's ability to early respond and efficiently mitigate the potential impact of introductions of devastating diseases, such as FMD. Here, disease transmission models have been used to predict the spread of FMD virus and evaluate the impact of control strategies. The objective of this project was to combine measureable information on FMD virus transmission into a disease model to measure the impact of movement data on the spread of FMD within a production system in the US to capture both within-herd and between-herd transmission. We aim to use these data to develop control strategies that incorporate currently accepted OIE containment strategies (i.e. movement bans, culling, and vaccination) and tailored control strategies based on characteristics of farms that may increase their risk of infection (i.e. number of shipments of animals received). The results of this study show that:

- Containment strategies significantly influence the predicted number of farms infected during an epidemic, as well as the mean duration of the epidemic. By increasing the radius of the control zones by 20% and 50%, we see a significant reduction in the predicted mean number of farms infected during an epidemic, as well as the mean duration of the epidemic.
- Moreover, FMD persistence within a population was dependent on farm structure and proportion of individuals in the population capable of becoming infected as seen in the modeled farrow to finish and farrow to wean herds, which are both birthing disease vulnerable piglets on a weekly basis.
- For those reasons, making the simplifying assumptions about how pigs contact each other on a farm may be sufficient to model FMD spread when aiming to measure average values of infection in the population. However, farm structure and demography needs to be considered to accurately model more detailed aspects of FMD infection in the herds.

Classical Swine Fever (CSF)

(09-111) Identification of host factors interacting with classical swine fever virus proteins: development of novel anti-viral therapeutics.

During the infection of a cell, a virus gets in contact with many host proteins. These interactions between virus and host factors enable the virus the successful production of progeny and progress of the disease. Identification and characterization of such interactions could be useful in providing novel alternatives to alter virus multiplication and, perhaps, disease. This project proposed the identification of swine proteins interacting with classical swine fever virus (CSFV) proteins during the infection. Results obtained enable the identification of several host proteins interacting with CSFV structural protein Core. Core protein is the major contributor to the virus capsid. Several of these interactions have been studied in detail and the regions of the CSFV Core protein interacting with the host proteins were identified. Mutant CSFV viruses having altered these regions have been demonstrated that have severely altered their ability to produce disease in swine. Therefore, the manipulation of the identified host-virus interactions allowed the development of attenuated strains of virus which may constitute a tool for the further development of live attenuated vaccine against classical swine fever. Additionally, this knowledge may open the possibility of designing bio therapeutic compounds that could alter those critical interactions that may limit the spread of the disease.

(11-001) Development of classical swine fever virus diagnostic assays for porcine oral fluid samples

With classical swine fever virus (CSFV) commonplace in the world and barely outside the borders of North America, it is reasonable to expect that CSFV will eventually be reintroduced into North America. The primary objective of this project is to optimize and validate technology capable of rapidly identifying premises infected with CSFV following its introduction into North America or other CSFV-free areas using oral fluid samples. In the present study, twenty pigs at the age of 21 days were obtained from a free-CSFV farm and divided into 3 groups. Pigs in group A (n=8) were intramuscularly inoculated with a low virulence CSFV strain (ALD, 105TCID50/pig) at day 0 and re-challenged with a virulence strain (Bangkok-1950, 103TCID50/pig) intramuscularly at 14 days post inoculation (DPI). Pigs in group B (n=8) were intramuscularly vaccinated with a commercial modified live CSFV vaccine (LOM) at day 0 and challenged with the virulence strain similar to pigs in group A at 14 DPI. Group C (n=4) was a negative control group. This modified experimental protocol allowed us to successfully collect the oral fluid samples from subclinically infected pigs and might mimic the CSFV situation in CSFV-endemic countries using modified live CSFV vaccines. Based on the modified real-time RT-PCR results (sensitivity at 101.5 TCID50/ml), a few vaccinated pigs having viremia for a few days after vaccination and after re-challenged with a high virulence strain, a few viremic pigs were found in both vaccinated and low virulence CSFV challenged groups. However, all samples were tested negative using a routine RT-PCR method (sensitivity at 103 TCID50/ml). In addition, none of the oral fluid samples from all groups were tested positive using either the modified real-time RT-PCR or the routine RT-PCR. Possible use of a commercial blocking ELISA of classical swine fever antibody was also evaluated by using oral fluid samples from a CSFV-free herd mixed with serially diluted known CSFV antibody titers as a pilot study and compared to the neutralizing peroxidase-linked assay (NPLA). Interestingly, the low levels of NPLA antibody titers (log2 1- log2 3) could evidently be detected in the oral fluid samples obtained from the in vivo experiment (lower than average serum NPLA titers of pigs in the same group, log2 3- log2 6) but none of the oral fluid samples from this present study were positive by a commercial ELISA (sensitivity at least log2 4 based on the pilot study). In conclusion, the modified real time RT-PCR yielded satisfactory sensitivity than the routine RT-PCR, but the sensitivity was not good enough to detect low virus levels, particularly in the oral fluid samples. Extracting genetic material from the oral fluid samples may be hampered by the mucous component in the saliva or other organic matters. Achievement of this objective could be done by developing a better genetic material extraction from oral fluid samples. However, detecting CSFV antibody in the oral fluid samples is another alternative method and when adjusting a commercial blocking ELISA protocol to increase its sensitivity for improved surveillance in CSFV-free areas, thereby would enhance elimination and control efforts. In addition, testing oral fluid samples using an improved sensitivity blocking ELISA may benefit the North American swine producers to rapidly identify premises infected with CSFV following its introduction into North America much faster than using the molecular diagnostic methods.

(11-045) Evaluation of envelope proteins for rapid induction of protective immune responses against classical swine fever

The main objective of this research project was to determine if native and/or modified forms of Classical Swine Fever Virus (CSFV) proteins present in the surface of the virion were able to induce a rapid and protective immune response against CSFV. To accomplish the proposed objective we took two approaches; 1) we assessed the capability each CSFV envelope proteins (E0, E1, and E2) for eliciting a protective immune response against the disease, and 2) we introduced modifications into CSFV envelope proteins to increase their capability of inducing an effective early protection against the virus. All three proteins (E0, E1, and E2) were produced using a baculovirus/insect cell system to obtain high protein yields. Proteins were purified, tested for purity, combined with adjuvant (Sigma), and used to immunize 30-40 lbs pigs. Animals were inoculated via IM receiving boosters at 28, 42, and 56 days post primo inoculation. All pigs were challenged intra-nasally (IN) 1 week after the last boost. Using this immunization scheme only those animals that were immunized with E0 or E2 elicited high antibody titers and survived the challenge. E1 protein did not elicit a detectable antibody response nor did it elicit a protective immunity in pigs against CSFV. All animals succumbed to challenge. To further understand the protective efficacy of E0 and E2 proteins we inoculated groups of 4 week-old swine with 1 or 2 doses of each protein. These animals were then exposed to CSFV IN at 21 days after single or last

inoculation. All the animals survived the infection, suggesting that lesser doses are sufficient to induce protection. Similarly animals receiving three doses of the proteins and challenged 1 week after last inoculation were protected against CSFV, suggesting that strong response can be induced shortly after 3 inoculations of both proteins. After the protective efficacy of wild-type E0 and E2 proteins was established, we introduced modifications to these proteins aimed to enhance the immune response against CSFV. E0 and E2 proteins were fused to flagellin; or to single chain anti-class II antibody sequences. Production and purification of modified proteins had to be adjusted from original procedures to obtain high yields of modified proteins. Proteins were then used to assess their capability to induce an antibody response in pigs. Unexpectedly, inoculation of these proteins into swine did not elicit a measurable antibody response suggesting that as formulated E0 and E2 will not induce an efficient immune response in pigs against CSFV. Work will continue to address this problem.

(14-087) Evaluation of diagnostic performance characteristics (sensitivity, specificity, onset of detection) of commercially-available CSFV tests

Classical swine fever virus (CSFV) is endemic and circulates in many regions of the world; therefore, the potential re-emergence of CSFV is a continual risk. It is in the pork producers' best interests to develop an effective CSFV detection-and-response strategy, recognizing that an effective response must be based on reliable technology capable of quickly identifying and eliminating foci of infection. The goal of this research was to evaluate the diagnostic performance characteristics of commercially-available CSFV tests. Samples used in this study were collected from pigs (n=30) intranasally inoculated with CSFV and from pigs (n=30) vaccinated with CSFV modified live vaccine. Following the CSFV inoculation, serum samples were collected on days post inoculation 0, 1, 2, 3, 4, 5, 6, 7, 10, 14, 17, 21, and 28. Serum (n=602) were tested by commercially-available CSFV assays including rRT-PCR (3 commercial assays), antigen-capture ELISAs (2 commercial assays), and antibody ELISAs (3 commercial assays). In addition, virus isolation (VI) and serum neutralization (SN) tests were also performed for comparison. The results from the present study indicated that each CSFV assay had its limitation(s), in large part depending on the test target (virus, antigen, nucleic acid, or antibody). Notably, commercial CSFV rRT-PCRs were more sensitive for early detection, whereas antibody assays were more sensitive in later stages. Therefore, it is important to perform the assay(s) most appropriate to the stage of infection (acute vs. chronic) and intended purpose (screening vs. confirmatory). Overall, commercial rRT-PCR and antibody ELISAs are suitable for large scale screening whereas virus isolation and serum neutralization should be used exclusively as confirmatory assays.

(15-176) Working toward a CSFV oral fluid antibody ELISA: defining oral fluid antibody (IgA, IgG) kinetics against envelope glycoproteins (E2, Erns)

Classical swine fever (CSF) is a highly contagious disease causing significant economic losses to swine producers in many part of the world. Although the US has been recognized as a CSFV-free area, effective tools for disease surveillance and monitoring for an active preparedness of this disease is required. Diagnostic assays based on oral fluid have been shown to be capable of excellence diagnostic performance, are extremely compatible with efficient surveillance, and are well-accepted by producers and veterinarians. The potential use of swine oral fluid as a sample for detection of CSFV infection was evaluated in this study. The study aimed to define oral fluid antibody kinetics (IgA, IgG) against two viral glycoproteins (E2 and Erns) using ELISA technique and to initiate the foundation to the logical development of a CSFV oral fluid antibody ELISA. The results showed that IgA and IgG antibody presented at detectable levels in oral fluid of both infected pigs and vaccinated pigs. Oral fluid IgG antibody provided strong and consistent detection over the time course of infection and vaccination. Compared to IgG responses, oral fluid IgA antibody showed lower and varied in the rate of detection. The results from the present study suggest a potential use of oral fluid antibody-based assays for detecting classical swine fever infection. The development of a sensitive and highly specific oral fluid CSFV antibody ELISA will be a key tool for cost-effective, large scale screening for CSFV. A CSFV oral fluid antibody ELISA would greatly improve the preparedness of the US Pork Industry.

(16-177) Development of Porcine Cytomegalovirus as a conventional and disseminating vaccine for Classical Swine Fever - A pilot study

Industry summary not available at time of publication.

African Swine Fever (ASF)

(11-022) Development of fluorescent recombinant antibodies to detect African Swine Fever virus in tissue samples and infected cells

The present project pretends to develop new reagents to solve an important gap in the African swine fever virus (ASFV) diagnosis. This virus is nowadays a real threat for Europe and, potentially, may spread to Asia or even other continents where the virus may produce a tremendous impact in the pig production. The serology of ASF has been resolved by the use of recombinant proteins in the diagnostic tests. These proteins have been validated and represent a better alternative to antigens obtained from infected cells (use of infectious virus for their generation). Recombinant antigens showed an improved sensitivity and specificity for antibody detection in chronically infected or unapparent carrier pigs and allowed the standardization of reagents production and tests interpretation. However, for the control or eradication of this important swine disease (no vaccine is available) is necessary to combine serology surveys with techniques for virus detection in samples of potentially infected pigs. Rapid detection means a minimization of disease spread risks to other animals or farms. Actually, the virus detection has to be done by PCR analysis (detection of viral DNA). This methodology detects accurately the virus presence in pig tissues, but needs a reference confirmatory technique because frequent false positive results, especially in laboratories with a reduced training level. The OIE recommend the virus isolation and the virus detection in animal tissues by antibody immunofluorescence. There are not available commercial universal reagents (antibodies) to carry out the immunofluorescence tests on tissue cuts or tissue explants. Additionally, the virus has to be isolated in primary pig macrophage cultures and, frequently, it takes several days and even weeks, depending of the virus titers in body fluids or organs, before the observation of the characteristic cytopathic effect or the haemadsorption reaction. The main objective of this project was to develop recombinant antibodies that could be used as reagent for sensitive detection of the virus in biological samples or infected cell cultures used for virus isolation. These antibodies, labeled with fluorescent molecules, will allow the virus detection using different technologies. These recombinant antibodies will avoid the use of sera from infected animals (potential risk of virus contaminations) or the use of monoclonal antibodies directed to variable epitopes of the virus that could fail in the detection of any specific virus strain. Antibodies would be produced by a cost-efficient system based on baculovirus vectors (a common system to produce biologics) and insect larva (living biofactories) instead insect cells. The larva system, only used for the moment by a reduced number of companies and research laboratories, is one of the most efficient and cost-effective system to produce any recombinant protein. These reagents (recombinant labeled antibodies) could be sent, without any risk, to reference diagnostic laboratories and would facilitate the standardization of results, independently of the expertise of professionals in ASF diagnosis. The limitation of the source of these antibodies would not be a problem for diagnostic laboratories in contrast to the limited source of antibodies obtained from immunized or naturally infected pigs. The conducted research during the granted 1 year project has generated different recombinant antibodies which are able to accurately detect ASFV in cell cultures. These antibodies were generated in insect larvae (IBES technology) with excellent productivities. Those useful reagents will be tested during the next months in samples from experimentally infected pigs to certify their sensitivity in virus detection and will also be tested in diagnostic laboratories from endemic regions (South Africa and Russia). Once those reagents were validated, those will be transferred to a company for their commercialization in any potentially affected country, including USA. Results obtained will also be published during the next months to disseminate the scientific information to the veterinary scientific community.

(12-106) Identification of genetic signatures for African Swine Fever virus serologic group specificity

African swine fever (ASF) is an acute viral hemorrhagic disease in domestic swine with mortality rates approaching 100%. Devastating ASF disease outbreaks and the continuing disease epidemic in the Caucasus region and Russia (2007 – to date) highlight the significance of this disease as arguably the most significant emerging disease threat for the swine industry worldwide. There is no vaccine for ASF available; however, it is clear that vaccination is possible since protection against homologous reinfection has been definitively demonstrated. Vaccine progress is hindered by lack of knowledge concerning the extent of ASFV strain variation and the viral antigens responsible for protective immunity. To date, eight ASFV serogroups have been identified although more likely exist. Notably and of great significance with respect to vaccine design and development, viruses within serogroups provide cross-protection from challenge with viruses within the serogroup. Unlike other viruses currently challenging the swine industry, such as PRRS, ASFV is a DNA virus and thus much less variable over time. Once the viral diversity currently circulating in natural reservoirs has been determined, a set of vaccines could be developed to be effective against relevant ASFV strains. Here, we have used a collection of serologically-grouped ASFV isolates and a large and diverse collection of ASF viruses to identify genetic signature(s) for ASFV serologic group specificity and to further define ASFV strain variability. We have demonstrated through gene sequencing and comparative analysis of ASFV strains a correlation between the genotype of the ASFV CD2v gene and virus grouping based on serospecificity. Overall, the concordance between CD2v region phylogenetic data and serogroup-specific typing provides predictive value of CD2v locus genotyping in predicting serologic, and potentially cross protective, virus groups. Results outlined here will have broad impact on vaccine-orientated approaches for ASF disease control thus reducing the threat posed by this high consequence viral disease. Knowledge of ASFV strain diversity and the breath of strain variation in nature as well as rapid genotyping methods to serotype viruses and to predict efficacy of a given vaccine to provide cross protection for a newly identified field isolate will facilitate vaccine design, development and emergency use.

(15-123) African Swine Fever Virus: a bioinformatics resource to support comparative genomics for vaccine and virulence studies

African swine fever is a very serious disease for which no vaccine is currently available. The virus (ASFV) has spread multiple times out of Africa into European countries and it is fortunate that it has not been introduced to North America where it would be disastrous for the pork industry. Vaccination is the only way to protect animals and eventually try to reduce the area in which the virus is endemic. To be able to create a vaccine that works with multiple genotypes of the virus, it is first necessary to determine the genetic sequence of all the types of virus. Fortunately, there are research projects planned by the ASFV research community to gather this information. The project described here was aimed at creating a database and bioinformatics resource to allow researchers to efficiently access and analyze ASFV genome information; specialized tools are required for viral genomes. The project's goals were achieved by modifying a database and tools that was originally designed for other large viruses (poxviruses). One of the key achievements was to enable the resource to reside in a computer cloud environment, which will allow the resource to be moved to other laboratories if required in the future. The database is now ready for the results of sequencing projects and it will enable researchers across the world to access the data and perform a variety of analyses.

(15-125) Rapid detection and epidemiological surveillance of African Swine Fever using oral fluid

Industry summary not available at time of publication

(15-126) Development of recombinant vaccinia virus vectored African Swine Fever vaccines

African swine fever (ASF) is an infectious and economically important disease of domestic pigs that poses a significant threat to worldwide swine industry including the US. Currently, there is no vaccine for ASF. In this proposal, 3 recombinant vaccinia virus vectored African swine fever vaccine candidates have been developed. Two different ASFV antigens were expressed in each recombinant virus. The immunogenicity of recombinant vaccine candidates was evaluated in pigs that were co-infected with 3 recombinant vaccine candidates. The results showed that an ELISA titer against ASFV antigen was detected in immunized pigs, indicating that the vaccine candidates are immunogenic in pigs. Nevertheless, ASF vaccine candidates have been developed and their efficacy need to be evaluated in pigs that will be challenged with a virulent ASFV when additional funding is available. If they are demonstrated to be able to protect pigs against the virulent ASFV challenge, they can be used as potential vaccines to protect the swine industry.

(16-007) Protective efficacy of an adenovirus-vectored ASFV multi-antigen cocktail

Industry summary not available at time of publication.

(16-021) Improving African Swine Fever diagnostics through the use of multi antigen print immunoassay (MAPIA) technology

Industry summary not available at time of publication.

(16-022) Evaluation of meat juice as a diagnostic sample for the detection of African Swine Fever

Industry summary not available at time of publication.

(16-184) Determination of the role of L83L, an uncharacterized ASFV protein that binds Il-1ß during ASFV infection

Industry summary not available at time of publication.

(17-057) Assessing the risk of African Swine Fever virus (ASFV) transmission in feed

Industry summary not available at time of publication.

Multi-viral Diagnostic Test Development and Validation

(14-284) Development and evaluation of a standardized protocol for utilization of swine oral fluids for PCR detection of high consequence pathogens: FMD, CSF, and ASF

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(14-286) Use of oral fluid samples to monitor virus shedding and antibody responses in pigs experimentally infected with high consequence swine viruses (Foot and Mouth Disease, African Swine Fever, Swine Vesicular Disease and Classical Swine Fever viruses)

Oral fluids (OF) obtained from pigs using cotton ropes are becoming a popular sample type for use in the diagnosis of diseases in pigs. The genetic material, antigens and antibodies to viruses that infect pigs can be measured in OF from pigs. Test methods exploiting this sample type are available for diseases frequently seen in North American pigs. Similar test methods need to be evaluated for diseases that are foreign or absent, but yet pose a threat to the North American pig industry. Examples include diseases caused by foot and mouth disease virus (FMDV), African swine

fever virus (ASFV), swine vesicular disease virus (SVDV) and classical swine fever virus (CSFV). This project was designed to partially address this need. Specific objectives were to create a repository of OF, swab and serum samples from FMDV, CSFV, ASFV and SVDV-infected pigs; use these samples to develop and/or validate existing test methods for detecting the genome of individual viruses (singleplex PCR) and multiple viruses simultaneous (multiplex assay) for FMDV, CSFV, ASFV and SVDV in OF; and develop and/or validate existing test methods for the detection of antibodies to FMDV, CSFV, ASFV and SVDV in OF and serum specimens collected during the convalescent phase of infection. For FMDV, groups of pigs were either directly inoculated intradermally in the heel bulb of one hind limb with cell culture supernatants containing FMDV or were inoculated by contact with the directly inoculated pigs. For SVDV, each pig was inoculated intradermally in the heel bulb of one hind limb with cell culture supernatants containing SVDV (4 groups of 4pigs /group). CSFV and ASFV inoculations were performed by administering virus to each animal through the nares and the mouth. Oral fluids were collected from each group of pigs using cotton ropes. Whole blood, serum and swabs of the mouth and nares were also collected from individual animals. FMDV genome was detected in OF as early as one day after the animals were either injected with virus or exposed to infected animals and 21 days later FMDV could still be detected in OF when tested by a quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Virus in these OF collected at 1-5 days post infection (DPI) grew in cell cultures, meaning infectious virus could be recovered from these samples. Slightly more virus was detected in OF compared to oral and nasal swabs. FMDV antigen was detected in OF by both a rapid penside test and an enzyme –linked immunosorbent assay (ELISA). Antibodies to FMDV were also detected in OF. The immunoglobulin (Ig) most commonly secreted at the mucosal surface is IgA. IgA was thus the most reliably detected antibody in OF in response to FMDV infection starting at 14 DPI and peaking at 21 – 28 DPI. SVDV genome was also detected in OF as early as one day after the animals were inoculated with virus and was still detectable at 21 DPI when tested by qRT-PCR. Similar to FMDV, SVDV in the OF collected at 1-5 DPI grew in cell cultures, meaning infectious virus could be recovered from these samples. Similarly, slightly more virus was detected in OF compared to oral and nasal swabs. OF was also a better sample type for SVDV detection when compared to serum. With a modified competitive ELISA based on commercially available monoclonal antibodies, antibodies to SVDV were detected in OF starting at 6 DPI. Antibodies of the IgM and IgA isotype were also detected in OF with IgM response starting at 6 DPI, reaching a maximum at 7 or 14 DPI and dropping at 21 DPI. The IgA response started at 7 DPI and peaked at 14 DPI. CSFV genome was detected in OF at 10 to 14 days after the animals were inoculated with virus. One group remained negative for virus genome in OF throughout the experiment. Virus was detected in sera earlier than in OF, starting at day 6 – 7 after inoculation of the pigs. Using a commercially available IDEXX HerdCheck CSFV Ab ELISA and a partially validated modification of the manufacturer's protocol, antibodies to CSFV were detected in OF starting at 14 - 21 DPI. ASFV Malta '78 genome was detected in OF starting at 6 DPI to 21 DPI. Detection of virus in oral and nasal swabs mirrored detection in OF. However, whole blood was the best sample type for ASFV detection, becoming positive at 4 DPI and containing higher levels of virus genome in most pigs. A fully integrated and automated assay for simultaneous detection and differentiation of FMDV, SVDV, CSFV and ASFV was developed. The fully integrated/automated assay was optimized, validated and used to successfully process and detect cell culture amplified viruses, as well as FMDV, SVDV, CSFV and ASFV in OF. All these results demonstrate that OF can be used for the detection of genome and/or live virus of FMDV, SVDV, CSFV and ASFV. Additionally, FMDV antigen can be detected in OF. Furthermore, antibodies to these viruses can be detected in OF by a variety of serological assays, including competitive and isotype-specific (IgA and IgM) ELISAs. Likewise, detection of IgA in OF has potential use for detecting antibody response following vaccination. All these results point to the high potential for the use of OF for FMDV, SVDV, CSFV or ASFV surveillance employing both established and partially validated assays.

(15-177) Development and validation of Singleplex and Multiplex Luminex Assays for Detection of antibodies to foot-and-mouth disease (FMD), swine vesicular disease (SVD), classical swine fever (CSF) and African swine fever (ASF) viruses in porcine oral fluids

Swine oral fluids (OF) are increasingly being used for the diagnosis of diseases in pigs. We have previously shown (NPB# 14-286) that the genome and antibodies to viruses that infect pigs can be measured in OF. The detection of

antibodies was mainly by enzyme-linked immunosorbent assay (ELISA), which might be less sensitive for OF than for serum. Luminex assays could potentially improve this sensitivity. The objective of this project was to develop and validate Luminex assays for detecting antibodies to FMDV, CSFV, ASFV and SVDV in OF. Specific objectives were to generate swine OF for test method development and validation through experimental inoculations of pigs with FMDV, SVDV, CSFV and ASFV; obtain samples from other members of the FAD Oral Fluid Consortium and laboratories in endemic countries; develop individual Luminex assays for detection of antibodies to FMDV, SVDV, CSFV and ASFV in swine OF; validate and compare method for antibody detection for the 4 viruses in OF and serum by singleplex and multiplex Luminex assays. Groups of pigs were either directly inoculated intradermally in the heel bulb of one hind limb with cell culture supernatants containing FMDV or were inoculated by contact with the directly inoculated pigs. For SVDV, each pig was inoculated intradermally in the heel bulb of one hind limb as well as through the mouth and the nares with cell culture supernatants containing SVDV (4 groups of 4pigs /group). CSFV and ASFV inoculations were performed by administering virus to each animal through the nares and the mouth. Oral fluids were collected from each group of pigs using cotton ropes. Serum and swabs of the mouth and nares were also collected from individual animals. Luminex assays were developed using recombinant 3ABC (FMDV), 3D (SVDV), Erns (CSFV) and p54 (ASFV) antigens produced in the baculovirus expression system. For multiplexing, the haemorrhagic diseases (ASF and CSF) and the vesicular diseases (FMD and SVD) were combined into duplex assays. At least 391 Samples from naïve animals as well as oral fluids from experimentally infected animals were tested to establish the specificity and sensitivity respectively for each assay. Oral fluids were also tested by previously optimized ELISAs to confirm the presence of relevant antibodies. Singleplex Luminex assays for the detection of antibodies to FMDV, SVDV, ASFV and CSFV in OF were developed and partially validated. Positive antibody detection for FMDV started as early as 4 days post infection (DPI). Positive antibody responses to SVDV 3D started at 14 DPI. Similarly positive antibody detection for ASFV and CSFV started at 10 and 14 DPI respectively. Comparable results were obtained in the duplex and singleplex assays. These results also mirrored those for sera from corresponding groups of animals. The data demonstrates that Luminex assays can be used for the detection of antibodies to FMDV, SVDV, CSFV or ASFV in OF. There is therefore a high potential for the use of OF for FMDV, SVDV, CSFV or ASFV surveillance using both Luminex assays and ELISAs.

(17-218) Negative cohort study plan for the estimation of diagnostic specificity of Two PCR assays for the detection of Classical Swine Fever, African Swine Fever and Foot and Mouth disease viruses in oral fluid samples (NCDA)

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(17-219) Negative cohort study plan for the estimation of diagnostic specificity of Two PCR assays for the detection of Classical Swine Fever, African Swine Fever and Foot and Mouth disease viruses in oral fluid samples (ISU)

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(17-220) Negative cohort study plan for the estimation of diagnostic specificity of Two PCR assays for the detection of Classical Swine Fever, African Swine Fever and Foot and Mouth disease viruses in oral fluid samples (SDSU)

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Epidemiology

(15-135) Characterizing a U.S. swine movement network to enhance epidemiological modeling and response to transboundary animal disease

The objectives of this project were to collect data that could be used to describe farm-to-farm swine movements in a major swine production region of the United States and to use the data to develop movement parameters that may be used in epidemiologic models of transboundary animal diseases. Epidemiologic models are simplified representations of disease spread in a population that are useful tools to study questions related to the characteristics of disease spread, the effectiveness of potential control strategies, and the effects of continuity of business activities during a simulated outbreak. Most epidemiologic models that have been used to simulate transboundary animal disease spread in the United States have focused on regions outside of the country's primary swine production regions. Consequently, swine have been underrepresented in those studies and have been described very generally in ways that do not adequately reflect the true structure and dynamics of U.S. swine systems. To create improved swine farm-to-farm movement parameters a dataset describing approximately 370,000 movements of animals in 17 primarily Midwestern states over one year was used to describe inter- and intra-state animal movements among four categories of farm (sow farm, finish, nursery, and wean-to-finish). In addition a dataset of approximately 9,000 movements of animals from Canada to the U.S. over one year were used to develop transboundary swine movement parameters between the two countries. Previously published epidemiologic models of transboundary animal diseases in the U.S. have tended to classify swine operations broadly as large (e.g., > 1,000 head) or small (e.g., < 1,000 head) operations and have not taken into account specialization in the industry and differences among types of operations in the destinations and types of animals that move among them. The movement parameters developed for this project will allow modelers to simulate movements among farms that better reflect the true structure and dynamics of U.S. swine systems.

Notes

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