Proposal for “Grow Iowa Values Fund” Grant Program

Prevention of Swine Influenza: Commercialization of Replicon Particle and Replicon Subunit Vaccines

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Dept of VMPM, 14 Kildee Hall, 515-294-5589, ryanvv@iastate.edu

Company Partner: Harrisvaccines, Inc. d/b/a Sirrah Bios, 17 employees, incorporated 2005, $1M plus annual sales. Contact: Jerry McVicker or Joel Harris, 2325 N. Loop Drive, Ames, IA 50010 515-296-3984

EXECUTIVE SUMMARY

Harris Laboratory has had two Grow Iowa Values Fund projects funded recently. The first project demonstrated replicon particles (RP) could induce an immune response in swine using a human influenza vaccine prepared by Alphavax (AVX). These were the first studies determining the effect of RP in pigs. The second project showed that RP technology can be useful in making a vaccine for prevention of porcine reproductive and respiratory syndrome (PPRS). Both of these studies, plus exclusive licenses from ISURF and AVX, have helped to create Harrisvaccines, Inc. d/b/a Sirrah Bios, a profitable Iowa company which employs 17 individuals at ISU Research Park facilities.

The AVX technology can be used to make both a replicon subunit (RS) and RP version of a vaccine. As a result Harrisvaccines operates as two entities. The Sirrah Bios division is currently selling a RS vaccine for PRRS under a Veterinary Client Patient (VCP) relationship (9 CFR §107.1) which does not require a USDA license, while the Harrisvaccines division is pursuing USDA licenses for RP vaccines for both PRRS and swine influenza virus (SIV) (H3).

Due to the occurrence of the 2009 Novel H1N1 influenza virus in humans and the numerous reassortant H1 viruses occurring frequently in swine, there is an urgent need for rapidly produced specific SIV vaccines which can protect against all these virus subtypes. The 2009 Novel H1N1 has caused over 15,000 infections and 99 deaths in humans thus far (May 29, 2009) and human to swine transmission has occurred in Canada. There are currently two additional H1 clusters of related SIV viruses occurring in swine in the U.S.: swH1β and swH1γ. Vaccination with one cluster subtype does not protect a pig against the other subtype. A vaccine prepared against the two current subtypes will not likely protect pigs against the 2009 Novel H1N1 and vice versa.

Currently available commercial swine vaccines do not protect against both the H1 subtypes and unlikely will protect against the 2009 Novel H1N1 virus. Because of the unique capability provided by AVX replicon technology, Sirrah Bios is in a unique position to quickly produce and sell RS vaccines for all subtypes of H1 and H3 influenza viruses. Simultaneously, the Harrisvaccines division can proceed with attaining USDA licensure for RP vaccines against all subtypes.
PROJECT DESCRIPTION

Technical Objectives
1. Create new RS and RP vaccines for 3 strains of SIV: 1) 2009 Novel H1N1 (A/California/04/2009), 2) swH1β, and 3) swH1γ
2. Evaluate immunogenicity of vaccines in pigs
3. Evaluate efficacy of vaccines in pigs in a vaccination-challenge model

Background and Technology
Swine influenza virus (SIV) is an RNA virus prone to mutation and recombination and thus is constantly changing (see Figure 1). Before 1997, there was only one subtype of SIV circulating in American swine herds, ‘classical’ H1N1. In 1997-98, there was a dramatic increase in seroprevalence and isolation of H3N2 SIV, leading to subsequent reassortant H1N1, H3N2, and H1N2 SIV subtypes. Currently, there are two clusters of H1 SIV circulating in U.S. swine: H1β (reassortant H1N1-like) and H1γ (H1N2-like). Current vaccines do not offer cross-cluster protection, and likely will not protect against the 2009 Novel H1N1 strain.

Figure 1. Epidemiology of SIVs in North America since 1918. Chronology of transmission events leading to reassortant viruses with genes from swine, human and avian influenza lineages. Swine virus lineage is color coded pink, avian lineage is coded green, human lineage is coded blue. Note, timeline not drawn to scale. From: Vincent AL, Ma W, Lager KM, Janke BH, Richt JA. Swine influenza viruses: a North American perspective. Adv Virus Res. 2008;72:127-54.

The AVX replicon technology uses the alphavirus Venezuelan Equine Encephalitis (VEE) virus as the basis for a vaccine vector. The technology involves the removal of components of the natural VEE virus, and replacement with an antigen from a targeted disease agent. Expression of this antigen induces an immune response against the disease of interest. The replicon technology platform merges subunit and live attenuated technologies providing a safe system of inducing protective immunity that mimics natural infection while producing numerous business advantages as well. Replicons can be created to express any antigen of interest (such as hemagglutinin (HA) from H1 or H3 viruses), thus making it an attractive candidate platform for influenza vaccination. With only a sequence of HA (rather than the entire virus), the HA gene can be synthesized in the laboratory. This gene is then inserted into the alphavirus replicon vector to produce
either RP or RS vaccines. This platform technology allows for rapid development and quicker turnaround time than traditional vaccines (weeks vs. months). Replicon particles express desired protein in vivo in the pig, whereas RS is made in vitro and adjuvanted protein is given as vaccine.

Our group has previous results demonstrating protection when pigs are vaccinated with the H3 RP. Vaccinated pigs had significantly lower viral loads detected in nasal swabs and BAL fluid than controls post-challenge. Gross lung lesions and body temperature were also lower in vaccinated pigs. Vaccinated pigs had high antibody titers as measured by HI assay, with mean titers peaking around 1:400. In a separate study, pigs vaccinated with a H3 RS vaccine developed HI titers ranging from 1:320 to 1:640 (≥1:40 is considered to be protective).

The importance of vaccination of swine against these endemic circulating SIV strains is highlighted by the recent outbreak of 2009 Novel H1N1 in humans worldwide. The studies proposed in this grant will provide evidence that both RP and RS vaccines are efficacious against circulating SIV strains, including the 2009 Novel H1N1

**Work Plan**

Replicons will be made expressing the HA genes from current H1 clusters currently circulating among US swine (H1β and H1γ) as well as 2009 Novel H1N1 strain. As stated, a replicon expressing triple-reassortant Clad IV H3 has already been made in our lab and shown to be protective as both a RP and RS vaccine. These replicons will first be evaluated for potential vaccine candidates based on an immunogenicity study. This first study will consist of 10 groups of 5 pigs each. Both RP and RS vaccine made from each replicon (H1β, H1γ, 2009 Novel H1N1, and H3) will be included, as well as commercial H1N1/H3N2 killed vaccine and negative control group. Immune response will be measured by the hemagglutinin inhibition (HI) assay, SIV ELISA, and western blot. For the HI assay, serum will be run against the other heterologous strains which were included in this study to see if there is cross-protection between H1 clusters and/or the 2009 Novel H1N1 strain. Titers obtained from this assay will also help determine challenge strain(s) to be used in the second study. If satisfactory results (HI titer ≥1:40) are seen the vaccines will be evaluated in a vaccination-challenge study.

Any challenge study using the A/California virus must be done in BL3 facilities, which are located at the National Animal Diseases Center in Ames, IA. All other challenge strains may be used in BL2 facilities located at Iowa State University. Assuming all vaccines induce significant HI titers, the challenge study design would be similar to the first immunogenicity study. For this study, however, there will be a total of 11 groups with 10 pigs each. Both RP and RS vaccine made from each replicon (H1β, H1γ, 2009 Novel H1N1, and H3) will be included, as well as commercial H1N1/H3N2 killed vaccine, negative and positive control groups. Caesarean-derived, colostrum-deprived (CDCD) pigs will be used in both of these studies to assure freedom from influenza virus infection and to avoid maternal antibody interference. Vaccine efficacy in the challenge study will be measured by live virus titration and qPCR from nasal swabs and tracheobronchial wash, body temperature, HI and ELISA titers, western blots, and gross lung involvement.
Commercialization Plan

The global market for animal vaccines is $2.5 billion. The swine portion is $475 million (19%) and expected to grow to $730 million by 2013. The growth is due to a stronger focus on prevention, rather than treatment of disease. This is similar to what has already occurred in the poultry industry where vaccines represent about 80% of all poultry animal health sales. In North America, distribution is aided by a highly consolidated market where 34% of pig production is under control of the top 5 producers and 50% is under control of the top 20.

The current economic impact of SIV to the entire pork industry is estimated to be $200 million per year, creating a significant opportunity for an efficacious SIV vaccine. Pfizer, Intervet (Schering-Plough), Newport labs, Novartis, and MVP all have commercially available vaccine products for SIV. All of these vaccine products utilize traditional inactivated killed whole virus. None of these companies are in the process of manufacturing vaccines using a replicon technology. Existing commercial vaccines may offer protection against one but not all SIV strains that are effecting pork producers today and may take a year or more to bring to market. It is important to note that SIV has mutated into multiple strains and will continue to mutate. The ability to react quickly and produce new vaccines is very important. Harrisvaccines / Sirrah Bios will be able to successfully commercialize the SIV vaccine because of:

- The speed to market provided by the VCP (Veterinarian / Client / Patient) relationship. This strategy is not used by large producers because of their use of the traditional distribution channel.
- The speed to large scale production provided by the AVX replicon technology.
- The exclusive license to AVX replicon technology which allows for multiple strain coverage in a single dose, as well as a “combo vaccine” approach, which would reduce labor by providing multiple disease coverage in a single dose as well.
- Highly competitive cost of production for both RS and RP vaccine versions.

Currently Sirrah Bios employs 48 veterinarians licensed in 21 states, in addition to our staff of 17 at our Ames, IA headquarters. The veterinarians are a pathway for producers to seek out solutions to their PRRS and SIV problems. Sirrah Bios helps the veterinarians solve these problems. Harrisvaccines is in the process of USDA licensure for the RP technology to be used in Swine. SIV-RP will be the first product licensed, however, because of the multiple strains and the lack of cross protection, multiple products would derive from this licensure.

Timeline (18 months)

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- Replicon synthesis/Vaccine production begins
- Grant funding begins
- Immunoergenicity Study
- Diagnostic testing and data analysis
- Challenge Studies (including Novel H1N1)
- Diagnostic testing and data analysis
- Manuscript and GIVF progress report preparation
## Budget (18 months)

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<th>CATEGORY</th>
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## Budget Justification

### Salaries

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### Benefits

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### Tuition

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### CDCD Pigs

(cost of sows, surgical derivation, and rearing until 6 weeks of age-see quote from Struve Laboratory)

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### Pig Per Diem (FY 2010)

160 pigs x $4.10/day x 49 days

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**Lab Supplies**
Replicon synthesis: $6,000 x 3 18,000  
(gene synthesis, lab consumables)
RP and RS Vaccine Production (media, roller bottles, sterility tests, potency assays)
Misc. Lab Supplies (syringes, needles, blood tubes, pipettes, tips, euthanasia drugs)

**Diagnostic Services**
HI assay: 25,600
160 pigs x 4 viruses x 5 bleedings/pigs x $8
SIV ELISA: 4000
160 pigs x 5 bleedings/pig x $5
Histopathology: 1600
160 pigs x $5/slide x 2 slides
In-house assays: 10,000
(Live virus titrations, qPCR, western blots)

**TOTALS** 146,610 77,169 72,770

**TOTAL COST-SHARE (CASH)** $149,939

**TOTAL AMOUNT REQUESTED** $146,610
Estimate

Ryan VanderVeen

Sows and C-Sections $ 36,618.00
Pigs to 6 wks of age $ 57,760.00
Study Events $ 1,584.00
Delivery $ 503.74

Subtotal $ 96,465.74
Tax
Total $ 96,465.74

Remittance Advice

Struve Laboratories
Attn: Jen
1603 Enterprise Street
Manning
IOWA 51455
(712)653-2125

Debtor:
Amount: $ 96,465.74
5/28/2009

Dr. Brad Bosworth
Iowa State University
Dept. of Animal Science
11 Kildee Hall
Ames, IA 50014

Dear Dr. Bosworth,

We are excited for this opportunity to evaluate the efficacy of vaccines for swine influenza virus as described in your grant proposal “Prevention of Swine Influenza: Commercialization of Replicon Particle and Replicon Subunit Vaccines”.

Harrissvaccines, Inc. agrees to supply in kind the replicon subunit (RS) and replicon particle (RP) vaccines as well as laboratory and technical support. The information gained from this project is crucial for further development of vaccines for prevention of respiratory diseases in swine.

We believe that the providing of efficacious vaccines for swine influenza virus will decrease the likelihood of pandemic influenza virus infections in humans.

Sincerely,

Jerry McVicker, PhD
COO
Harrissvaccines, Inc.

Joel Harris
COO
Sirrah Bios
PI Name: Brad Bosworth

Project Title: Prevention of Swine Influenza: Commercialization of Replicon Particle and Replicon Subunit Vaccines

College Ranking _2 of 6_

Recommendation: Fund _X__ Fund if Possible ___ Do Not Fund ___

SECTION I: TECHNICAL MERIT (60% of recommendation)

Low  5

Considerations:
- What is the scientific merit of the proposed project?
- Is the project technically feasible to accomplish in the listed time frame?
- Does the budget seem reasonable?
- Does the PI/team of researchers have the qualifications necessary to carry out the work?

Justification:
High scientific importance with a highly qualified team of researchers. Modest and appropriate budget for a 1.5-yr project. A slight concern about the ability to use the BL3 facilities at the NADC for a key challenge study.

SECTION II: BROADER IMPACTS (30% of recommendation)

Low  4.5

Considerations:
- What is the probability that this project could be used to leverage future funding from non-ISU sources?
- What is the potential to increase ISU's research capabilities or capacity?
- What is the potential to enhance learning opportunities for students?

Justification:
Strong industry connection. Clear leveraging of industry-sponsored research. Unclear connection with graduate students.

SECTION III: COMMERCIAL POTENTIAL (10% of recommendation)

5

Considerations:
- What is the likelihood that new intellectual property will be generated?
- What is the likelihood of eventual commercial success?

Justification:
High likelihood of new IP and eventual commercial success.
**Proposal Number:** FY10-5  
**PI Name:** Brad Bosworth  
**Project Title:** Prevention of Swine Influenza: Commercialization of Replicon Particle and Replicon Subunit Vaccines  
**College Ranking:**  
**College Rating:**  
**Commercial Recommendation:** Fund

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**Commercial Potential:**

1) Will this project lead to a new Iowa business/company, or increase the sales/profitability of a recently started Iowa company?

   **Yes**


2) Will this project increase the sales or profitability of an existing Iowa business/company?

   **Yes**

   What is the probability of commercial success: Speed new products-Swine influenza virus vaccines-to market. SIV is huge problem for swine industry. Novel H1N1 virus poses new threat of pigs getting sick from exposure to infected humans.

3) Are competitors identified, is the advantage of the proposed technology clear.

   **Yes**

   Please comment on the above: Traditional vaccines work against only one strain of SIV. Replicon technology would allow several strains to be incorporated into the same vaccination. Exclusive license from AlphaVax to use replicon technology for use in pigs provides barrier for competitors to develop similar vaccines.

4) Is there a clear strategy for entry to the market, start up or existing Iowa business/company.

   **Yes**

   Please comment on the above: The V/C/A relationship will allow quick response to mutating viruses. Application for USDA licensure of RP vaccine for SIV in process. Current application would cover multi-strain SIV vaccines developed under project.

5) Please make any other comments related to the commercial potential of this proposal: Proposal addresses timely problem for Iowa and swine industry. Current economic impact of SIV is ~$200M/year. Good need for product. Company doing well; cool IP

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**Intellectual Property Evaluation**
1. Have any current ISURF invention disclosures been identified as background IP? **No**

   **The company has background IP and license from AlphaVax for key background patents.**

   **Strong IP position.**

   If Yes:

   a) What is/are the ISURF number(s)?

   b) What is the IP/patent status?

   c) What is the licensing status?

2. Does this project have the potential to generate new intellectual property? **Yes**

   (please explain) **Likely joint between Harrisvaccine/ISU.**

3. Based on your current knowledge, without having the opportunity to receive input from the principle investigator or to conduct a market or technology assessment, what will be the barriers to commercializing this technology?