Mission is to conduct basic and applied research on selected diseases and food safety pathogens of economic importance to the US livestock and poultry industries
Vaccine Research at NADC

Brucellosis
Tuberculosis
Spirochaetal Diseases – Leptospirosis and digital dermatitis
Johne’s Disease (*Mycobacterium avium* subsp *paratuberculosis*)
*Mannheimia haemolytica*
*Pasteurella multocida*
*Mycoplasma bovis*
Bovine Respiratory Syncytial Virus
Bovine Viral Diarrhea Virus
PRRSV
SIV
PEDV
Senecavirus A
*Salmonella*
*Campylobacter jejuni*
*Streptococcus suis*
*Haemophilus parasuis*
Biotechnology Role in Disease Control

Only 2 diseases eradicated through human efforts

- Smallpox – WHO in May 1980
- Rinderpest – FAO in Oct 2010 & OIE in May 2011

Both efforts benefited from live vaccines
Brucella Vaccines

- Vaccines are good at preventing clinical effects (abortion) and transmission; not infection or seroconversion
- Vaccination alone will not eradicate disease
- Species differences in immunologic responses
- Requirement for remote vaccine for wildlife species
- Disease in swine different from ruminant brucellosis
Booster Vaccination with RB51

• In bison and cattle, significantly reduces abortion and infection as compared to single vaccination
• Reduces CFU/gm of tissue and therefore disease transmission
## Efficacy of RB51 in Bison - Overall Data: 2016

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Abortion</th>
<th>Fetal/Mam. Infection</th>
<th>Maternal Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>70</td>
<td>87% (91/70)</td>
<td>91% (64/70)</td>
<td>100% (70/70)</td>
</tr>
<tr>
<td>Hand RB51</td>
<td>83</td>
<td>32% (27/83)*</td>
<td>48% (40/83)*</td>
<td>81% (67/83)</td>
</tr>
<tr>
<td>Booster RB51</td>
<td>19</td>
<td>5% (1/19)*</td>
<td>37% (7/19)*</td>
<td>74% (14/19)*</td>
</tr>
</tbody>
</table>

* Significantly different (P < 0.05) than Control
Distribution of Feral Swine in the US

GPS mapping at http://128.192.20.53/infsms/
Efficacy of 353-1 to protect swine against *B. suis*

<table>
<thead>
<tr>
<th>Control</th>
<th>Parenteral Vaccinates</th>
<th>Oral Vaccinates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feral Swine (intact male and female)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major organs</td>
<td>9/9</td>
<td>0/5</td>
</tr>
<tr>
<td>Reproductive tissues</td>
<td>8/9</td>
<td>0/5</td>
</tr>
<tr>
<td>Cervical lymph nodes</td>
<td>7/9</td>
<td>0/5</td>
</tr>
<tr>
<td>Other lymph nodes</td>
<td>9/9</td>
<td>0/5</td>
</tr>
<tr>
<td><strong>Domestic Swine (barrows)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major organs</td>
<td>0/7</td>
<td>0/6</td>
</tr>
<tr>
<td>Cervical lymph nodes</td>
<td>6/7</td>
<td>3/6</td>
</tr>
<tr>
<td>Other lymph nodes</td>
<td>3/7</td>
<td>1/6</td>
</tr>
</tbody>
</table>
Vaccine Studies with White-tailed Deer

• Orally administered BCG decreases disease severity in white-tailed deer.
• BCG is safe for deer.
• BCG can persist in tissues for up to 12 months after vaccination.
• Vaccinated deer can shed BCG that is then found in sentinel cohorts (deer).
• BCG is not readily shed from vaccinated deer to cattle (sentinel cattle do not develop positive skin test or IFN-γ responses).
Research Gaps

Cattle

• Will BCG plus mucosally-delivered subunit vaccines (i.e., *Mannheimia* expressing Ag85 and TB10.4) provide improved protection and longer duration of immunity than BCG alone?

White-tailed Deer

• Does decreased disease severity equate to decreased transmission?
• What is the duration of immunity?
• Does secondary vaccination (exposure through vaccine shedding) provide protection.
Paratuberculosis Vaccine Research
Judith Stabel, PhD & John Bannantine, PhD

- USDA-licensed vaccine in US
  - Mycopar – bovine strain
  - Whole cell heat-killed product in oil
  - Vaccinate neonates at < 1 month of age
  - Vaccinate in brisket due to granulomatous lesion
- International vaccines
  - Gudair (sheep/goats);
  - Silirum (sheep/goats/cattle)- strain 316F (bovine)
Recent Novel Vaccine Candidates

Mycobacterial 70 kD heat-shock protein is an effective subunit vaccine against bovine paratuberculosis

Ad Koets a,b,*, Aad Hoek b, Merel Langelaar a,b, Marije Overdijk b, Wiebren San tema b, Peter Franken c, Willem van Eden b, Victor Rutten b

Clinical and Vaccine Immunology

Evaluation of a Mycobacterium avium subsp. paratuberculosis leuD Mutant as a Vaccine Candidate against Challenge in a Caprine Model

Syed M. Faisal, Jenn-Wei Chen, Falong Yan, Tsai-Tzu Chen, Nicodemus M. Useh, Weiwei Yan, Shuangang Guo, Shih-Jon Wang, Amy L. Glaser, Sean P. McDonough, Bhupinder Singh, William C. Davis, Bruce L. Akey and Yung-Fu Chang

Attenuated strains of Mycobacterium avium subspecies paratuberculosis as vaccine candidates against Johne’s disease

Erik W. Settles a,b, John A. Kink a, Adel Talaat a,b,c,*

Evaluation of novel oral vaccine candidates and validation of a caprine model of Johne’s disease

Murray E. Hines II*, Sue E. Turnquist †, Marcia R. S. Ilha †, Sreekumari Rajeev †, Arthur L. Jones ‡, Lisa Whittington †, John P. Bannantine †, Raúl G. Barletta †, Yrjö T. Gröhn ‡, Robab Katani ‡, Adel M. Talaat ‡, Lingling Li ‡ and Vivek Kapur ‡
Problems with Vaccines

- Granulomatus lesions at injection site
- Cross-reactivity with *M. bovis* causing misdiagnosis of animals
- Does not prevent infection; just reduces clinical signs
Whole Blood IFN-γ Responses to MAP Proteins

**Fig. 1.** Secretion of interferon-γ (Abs \(_{450\text{nm}}\); IFN-γ) by control noninfected cows and cows naturally infected with *Mycobacterium avium* subsp. *paratuberculosis* upon incubation of whole blood with medium alone (NS); concanavalinA (ConA); pokeweed mitogen (PWM); a whole-cell sonicate of *Mycobacterium avium* subsp. *paratuberculosis* (MPS); johnin purified protein derivative (JPPD); and MAP proteins (2077c, 1204, 1272c, and 1087). Data are expressed as means ± SEM. Significant differences between control and infection cows within in vitro treatment group are represented by asterisks (\(^*\) *P* < 0.01; \(^*\) *P* < 0.05).
Application of Protein Cocktail as Vaccine in Calf Model

- Used all 4 MAP proteins
  - Equivalent amounts of each protein in cocktail
    - Yield 100 μg each protein and 500 μg DDA adjuvant per dose
    - 1 ml dose subcutaneously admin
    - Booster at 2 weeks
    - Challenge with live MAP (10^8 cfu/inoculation) in milk replacer at days 0, 3, 7
    - 12 month study; sampling early followed by 3 month periods; necropsy at termination
Application of Protein Cocktail as Vaccine in Calf Model
All 4 proteins used in cocktail

Tissue colonization – CFU’s
**P < 0.01 All tissues
Application of Protein Cocktail as Vaccine in Calf Model

Fecal Shedding of MAP

Days after Inoculation

Days: 0, 7, 14, 30, 90, 180, 270, 360

cfu/g feces: 0, 10000, 20000, 30000, 40000

Non-Vacc
Vacc
Follow-up Study of Protein Vaccine in Calf Model currently underway

- One cocktail of all 4 proteins
- Two doses + control infected (n = 5)
  - 100 µg each protein
  - 200 µg each protein
  - DDA adjuvant
  - Subcutaneous admin/1 ml; booster at 2 weeks
  - Inoculate with live MAP on days 0, 3, 7
  - Sample calves as before; planned 12 month
Salmonella vaccine
Challenging!

>2,500 serovars
Ubiquitous in nature
Wide host range
Gastrointestinal to Systemic disease
Livestock are often asymptomatic carriers
Limitations of current *Salmonella* vaccines

Seroivar-specific protection

Vaccination may interfere with *Salmonella* surveillance programs to identify *Salmonella*-positive herds or flocks.
Attenuated *S. Typhimurium* strain

Reduction in disease and colonization

Cross-protection against multiple serovars

DIVA (Differentiation of Infected from Vaccinated Animals)

---

Reduce the variable, immunodominant *Salmonella* antigens (LPS and flagella)

Increase conserved, *Salmonella* outer membrane proteins
General properties of *Salmonella* Typhimurium BBS 866 live vaccine:

- No significant increase in body temperature (fever) following vaccination of pigs or turkeys (i.e. vaccine is attenuated)
- Differentiation of Infected from Vaccinated Animals (DIVA); vaccine strain is not detected in pigs by IDEXX HerdCheck Swine *Salmonella* Test Kit
Salmonella Vaccine Trials

Swine *Salmonella enterica* serovar Typhimurium challenge study:

- No significant increase in body temperature following wild-type challenge for vaccinated pigs (significant increase occurred in mock-vaccinated pigs)
- Significant 100-fold reduction in fecal shedding for vaccinated compared to mock-vaccinated swine
- Significant 10-50 fold reduction in gastrointestinal tissue colonization for vaccinated compared to mock-vaccinated swine
Swine *Salmonella enterica* serovar Choleraesuis challenge study:

- Vaccination reduced duration and degree of fever
- Vaccination affected average daily gain of Choleraesuis infected pigs: ABW increased in vaccinated pigs and decreased in mock-vaccinated pigs
- Vaccination significantly decreased Choleraesuis bacteremia
- Significant 100-fold reduction in liver, spleen and tonsil colonization for vaccinated compared to mock-vaccinated swine
- Significant 10-100 fold reduction in gastrointestinal tissue colonization for vaccinated compared to mock-vaccinated swine
Salmonella Vaccine Trials

Turkey *Salmonella enterica* serovar Heidelberg challenge study:

- Vaccination significantly decreased the number of Heidelberg-positive spleens
- Significant 10-50 fold reduction in ceca colonization for vaccinated compared to mock-vaccinated swine
Salmonella Typhimurium BBS 866 live vaccine

Attenuated in pigs & turkeys

Differentiation of infected from vaccinated animals (DIVA)

Cross-protective against wild-type challenge with Cholerasuis (pigs) and Heidelberg (turkeys)

Modified-live bacterial mucosal vaccines for diseases of beef and dairy cattle.

Robert E. Briggs and Fred M. Tatum
National Animal Disease Center, Ames, IA, USA.
**Mannheimia haemolytica**

- *M. haemolytica* - commensal which colonizes tonsils and nasal passages of cattle, sheep, and goats.
- Few isolations from healthy, unstressed calves on farm and order-buyer barn.
- Many isolations in high numbers at feedyard after transport.
- Readily spreads among stressed and non-stressed calves.
- Colonization elicits local and systemic immune response and resistance to further colonization.
- Once colonized, calf tends to retain same strain for duration of colonization.
**Pasteurella multocida**

- *P. multocida* - commensal which colonizes tonsils and nasal passages of cattle, sheep, and goats.
- Colonization unaffected by stress or viral infection.
- Colonization elicits local and systemic immune response and resistance to further colonization.
- Highly opportunistic, pneumonia is strongly associated with prior pulmonary insult such as by virus or *M. haemolytica*.
- Capsule type A, serotype 3 most common.
Modified-live bacterial vaccines

- Broader cell-mediated and mucosal immunity over killed or soluble preparations.
- Single exposure for active immunity.
- Rarely cause hypersensitivity reactions which can occur with killed preparations.
- Capable of induced expression of stress proteins and certain toxins within the host.
- Stable, genetically-defined attenuated bacterial strains can be constructed.
Construction of modified-live bacterial vaccine candidates

- Identify gene relevant to virulence
- Clone gene and flanking DNA
- Design deletion to inactivate gene
  - If gene relevant to immunity, leave immunogenic portion intact
- Introduce gene into organism on ts plasmid
- Screen for desired product
Leukotoxin operon:

lktC - acylates leukotoxin structural gene to activate
lktA - leukotoxin structural gene
lktB/D - involved in leader-independent leukotoxin export
Common promoter for entire operon
**M. haemolytica lktCA**

3.15 kb

Digest *NaeI*, ligate.

1035 bp (345 aa) deletion
Western blot of native leukotoxin and ΔLktA using anti-Lkt monoclonal antibody.
Effect of modified leukotoxin gene

Lung lesions

- Control
- Oral
- Injectable

Lung bacteria

- Control
- Oral
- Injectable

Billions

- $1.00E-02$
- $1.00E-03$
- $1.00E-04$
- $1.00E-05$
- $1.00E-06$
- $1.00E-07$
- $1.00E-08$
- $1.00E-09$
Efficacy of injectable $\Delta lktA$ modified-live in sheep and goats.

<table>
<thead>
<tr>
<th></th>
<th>Control$^1$</th>
<th>Vaccinate$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=5</td>
<td>n=5</td>
</tr>
<tr>
<td>Lung lesions</td>
<td>40%</td>
<td>2%$^*$</td>
</tr>
<tr>
<td>IHA titer St5:St6</td>
<td>6:3</td>
<td>73:111$^{**}$</td>
</tr>
<tr>
<td>Lkt neut. titer</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>Lung bacteria</td>
<td>$10^{7.8}$</td>
<td>$10^{1.1}$</td>
</tr>
</tbody>
</table>

$^*$P<0.001  
$^{**}$ Little or no response to second vaccination

$^1$ 1 sheep and 3 goats died within 2 days of challenge, 1 sheep was severely ill
$^2$ All animals remained afebrile and on-feed
Field trials of mucosal vaccines in transported beef calves

- Tested were *Mannheimia*-only and *Mannheimia/Pasteurella* combined
- n=84 to 220 calves per study balanced for vaccinated and control
- Vaccine delivered single dose on feed or intranasal
- Vaccine delivered point-of-first-assembly or at experimental feedlot
- Calves monitored for approximately first 5 weeks on feed

- Delivery at point-of-first-assembly enhances weight gain
- Increased serum titer in vaccinates
- Reduced infectious load of *Mannheimia*
- Reduced morbidity and re-treats
Summary

• Modified-live vaccine strains:
  - Produce complete antigen repertoire
  - Colonize upper-respiratory tract and tonsils
  - Not pathogenic in domestic animal lung tissues
  - Elicit local and systemic antibody and memory
  - Single dosage appears to be sufficient

• Vaccine is effective after simple top-dressing on feed (calves)
  - Eliminate injection-site reactions
  - Easy delivery / Avoid stress of animal restraint

• Greatly reduces or eliminates clinical signs and lung lesions in laboratory trials

• Polyvalent vaccine is effective
Modified-live bacteria are fast and effective when delivered on mucosa…

Important bovine pathogens’ port-of-entry is often the naso-pharynx or oro-pharynx…

What if heterologous immunogens were delivered carried and expressed by such bacterial vaccines?

*Brucella abortus*
*BVDV*
*Moraxella bovis* (pinkeye)
*Mycobacterium bovis*
*Mycoplasma bovis*
Diversity of swine IAV shaped by human IAV


T.K. Anderson
How do we know what’s out there?

Surveillance Pyramid

- Whole Genome Sequencing
- Sequencing HA/NA and Genetic Characterization
- PCR Detection/Subtyping

ANIMAL STUDIES
ANTIGENIC ANALYSIS
VIRUS REPOSITORY

Monitor genetic and antigenic evolution
Inform animal and public health stakeholders
Update diagnostics reagents and vaccines
Identify relevant strains for research
Development of intervention strategies

Surveillance of Influenza in Swine

USDA
NADC
National Animal Disease Center
ISU-VDL Frequency of IAV as a Respiratory Disease Diagnosis for Swine

Percent of Respiratory Diagnosis

- PRRSV: 43.8%
- IAV-S: 24.6%
- M. hyopneumoniae: 7.1%
- P. multocida: 6.5%
- H. parasuis: 6.3%
- Bacterial: 2.8%
- A. suis: 2.1%
- B. bronchiseptica: 1.6%
- APP: 1.6%
- A. pyogenes: 1.0%
- Idiopathic: 0.9%
- PCV: 0.7%
- Viral: 0.6%
- Salmonella: 0.3%
- PRCV: 0.1%
- Parasitic: 0.0%

2015

Phil Gauger, ISU VDL
USDA IAV Swine Surveillance

• USDA APHIS Veterinary Services system, active since 2009
• Virus isolates have HA, NA and M sequenced for all, WGS for subset
• Sequences in GenBank and isolates available through USDA NVSL repository
  • A/swine/Arkansas/A01840698/2015
  • Email your request to: NVSL_Userfee@aphis.usda.gov

NADC does genetic, antigenic, and phenotypic characterization on viruses of interest
Flu Vaccine Basics

• HA is the major vaccine target
  ✷ Robust hemagglutination inhibition (HI) antibody titers as gold standard
  ✷ Antibodies to receptor binding site and surrounding ridge can be neutralizing

• NA is a secondary vaccine target
  ✷ Antibody response measured by neuraminidase inhibition (NI) assay
  ✷ Reduces progeny virus egress and decreases shedding/transmission

• Other “internal” gene proteins included in whole virus products
  ✷ Antibody detection for diagnostic purposes (NP)
  ✷ T-cell immunity
Antigenic drift over time

Why vaccines may fail

- Poorly matched HA and NA antigens
- Antigenic load and balance in multivalent vaccines
- Poorly immunogenic antigens
- Not all adjuvants are the same
- Original antigenic sin
- Back-boosting
- Antigenic imprinting
- Maternal antibody interference with vaccine
- VAERD: oil-in-water adjuvanted WIV, HA protein subunit, and maternal derived antibody

- Pre- and post-vaccination serum from vaccinates should be tested against each of the vaccine antigens in the HI assay to understand if the issues above are at play!

Future Opportunities

- Surveillance data revealing patterns from which to draw vaccine strain decisions
- Changes in licensing regulations
- New vaccine platforms and technology
  - Rapid change of HA and NA
  - Broader cross-protection (LAIV>Vectored>WIV)
  - Improved mucosal immunity
- New computational tools to enhance surveillance and HI data analysis
  - Allowing a better understanding of antigenic consequence of changes in the HA
A protein subunit vaccine provides significant protection against virulent *Streptococcus suis* in pigs.

Susan L. Brockmeier, Crystal L. Loving, Tracy L. Nicholson, Jinhong Wang, Sarah E. Peters, David J. Seilly, Paul R. Langford, Andrew Rycroft, Brendan W. Wren, Alexander W. Tucker, Duncan J. Maskell on behalf of the BRADP1T Consortium
Streptococcus suis

- Gram-positive bacterium commonly carried in the tonsil and nasal cavity of swine
- Streptococcal disease is widespread wherever pig production occurs and systemic invasion causes septicemia, meningitis, arthritis, and/or polyserositis
- S. suis is also a zoonotic agent capable of causing meningitis in humans
- There are at least 35 capsular serotypes
  - S. suis and capsular serotype 2 is the most virulent and the most frequently isolated from both diseased swine and humans
  - Depending on geographic location other serotypes such as 1, 1/2, 3, 7, 8, 9, 14 are commonly isolated from diseased pigs
- Due to the presence of multiple serotypes and high genotypic variability, efficacious vaccines are not readily available and need to be developed
Selection of candidate vaccine proteins

- TraDIS (Transposon Directed Insertion Sequencing), method to identify bacterial fitness genes, used with an \emph{in vitro} organ culture system (IVOC) then combined with \emph{in silico} bioinformatics approaches to identify 5 proteins of \textit{S. suis} predicted to be important for colonization of the respiratory tract of swine.
  - Candidate fitness genes were determined - if a gene harbored at least one transposon insertion mutant with significant reduction in fitness in a respiratory epithelium IVOC system
  - Protein subcellular localization was predicted \emph{in silico} with bioinformatics approaches to shortlist fitness genes encoding surface-associated proteins
  - \emph{In silico} protein homology based searches were used to identify proteins with cross-protection potential
Screen for mucosal survival of mutants using *In vitro Organ Culture (IVOC)*

- Whole slices of trachea, turbinate, or nasopharynx
- Maintained at an air interface
- Mucociliary function maintained
- More physiologically relevant than submerged culture
- Replaces large numbers of animal experiments

[Diagram showing Tissue explant, Agar plug, Filter paper, Liquid media]
Mutations affecting fitness for colonization

Input pool of random transposon mutants

Massively parallel sequencing of genomic regions flanking transposons (TraDIS)

Map sequence reads to genome and compare input and output counts

Relative fitness: Intermediate Low High

Roy Chaudhuri
## Presence of five immunogenic antigens in 459 isolates of *S. suis*

<table>
<thead>
<tr>
<th>Protein</th>
<th>No. of isolates in which protein is present</th>
<th>Clinical (292 isolates)</th>
<th>Non-clinical (134 isolates)</th>
<th>Not Known (33 isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>459</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>B</td>
<td>452</td>
<td>99%</td>
<td>97%</td>
<td>94%</td>
</tr>
<tr>
<td>C</td>
<td>459</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>D</td>
<td>450</td>
<td>98%</td>
<td>97%</td>
<td>100%</td>
</tr>
<tr>
<td>E</td>
<td>458</td>
<td>100%</td>
<td>99%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Protein identities of the five subunit vaccine candidates in disease-associated *S. suis* serotype representatives

<table>
<thead>
<tr>
<th>Serotype</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100%</td>
<td>99.5%</td>
<td>100%</td>
<td>100%</td>
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</tr>
<tr>
<td>1/2</td>
<td>99%</td>
<td>99.5%</td>
<td>99.5%</td>
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<td>2</td>
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<td>7</td>
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</tr>
<tr>
<td>8</td>
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<td>97.5%</td>
<td>100%</td>
</tr>
<tr>
<td>9</td>
<td>97%</td>
<td>90.5%</td>
<td>96%</td>
<td>96%</td>
<td>97%</td>
</tr>
<tr>
<td>14</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
## Vaccine study design

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccine/Adjuvant/Route</th>
<th>Challenge</th>
<th>Number of Pigs</th>
</tr>
</thead>
</table>
| Group 1 | *S. suis* proteins/Polyethyleneimine/IN  
*S. suis* proteins/Carbopol® & AddaVax™/IM                                           | *S. suis* P1/7 | 6              |
| Group 2 | *S. suis* proteins/ Polyethyleneimine/IN  
*S. suis* proteins/Emulsigen®D/IM                                                         | *S. suis* P1/7 | 6              |
| Group 3 | PBS/ Polyethyleneimine/IN  
PBS/ Carbopol® & AddaVax™/IM                                                               | *S. suis* P1/7 | 3              |
| Group 4 | PBS/ Polyethyleneimine/IN  
PBS/ Emulsigen®D/IM                                                                              | *S. suis* P1/7 | 3              |
| Group 5 | PBS/none/IN  
PBS/none/IM                                                                                      | *S. suis* P1/7 | 4              |

Pigs vaccinated twice 2 weeks apart, and 2 weeks later challenged with 2 ml of $10^9$ CFU/ml *S. suis* (P1/7) IN.
Subunit vaccination provides significant protection against lethal challenge with *S. suis* and correlates to immune response and adjuvant
Subunit vaccination induces IgG reactive against whole *S. suis* bacteria and cross reactive antibodies to other strains.
Summary

- All 5 proteins predicted to have functions in several physiological processes explaining role in *S. suis* survival on respiratory epithelia.
- All 5 proteins are good immunogens as robust antibody responses were raised to each.
- *In silico* bioinformatics approach indicated genes encoding surface-associated proteins were highly conserved.
- Reactivity of immune sera against several *S. suis* serotypes indicates potential for cross-protection.
## New Emerging and Re-emerging Animal Diseases

An incomplete and growing list of diseases of past 3 decades

<table>
<thead>
<tr>
<th>Year</th>
<th>Disease/Location</th>
<th>Year</th>
<th>Disease/Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>BSE - England</td>
<td>1986</td>
<td>PRRSV</td>
</tr>
<tr>
<td>1994</td>
<td>Hendra virus</td>
<td>1995</td>
<td>Tuberculosis - WTD</td>
</tr>
<tr>
<td>1997/98</td>
<td>H3N2 SIV w/TRIG</td>
<td>1998</td>
<td>Leptospirosis - triathletes</td>
</tr>
<tr>
<td>1998</td>
<td>Nipah virus; EHDV in deer</td>
<td>1990’s/2004</td>
<td>Hobi-like pestivirus</td>
</tr>
<tr>
<td>1999</td>
<td>West Nile Virus in U.S.</td>
<td>2003</td>
<td>Monkeypox virus</td>
</tr>
<tr>
<td>2003</td>
<td>SARS virus</td>
<td>2003</td>
<td>HP H5N1</td>
</tr>
<tr>
<td>2003</td>
<td>1st U.S. BSE (classical)</td>
<td>2004</td>
<td>Canine H3N8</td>
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<td>2004</td>
<td>BSE (atypical sporadic) in U.S.</td>
<td>2005/6</td>
<td>PCVAD</td>
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<td>2006</td>
<td>BSE (atypical genetic) in U.S.</td>
<td>2006</td>
<td>HP Asian PRRSV &amp; BTV</td>
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<tr>
<td>2008</td>
<td>Reston Ebolavirus in swine</td>
<td>2009</td>
<td>pandemic H1N1</td>
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<tr>
<td>2010-present</td>
<td>H3N2v</td>
<td>2011</td>
<td>Schmallenberg Virus</td>
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<tr>
<td>2012</td>
<td>BSE (atypical sporadic) in U.S.</td>
<td>2012/2013</td>
<td>MERSV &amp; H7N9</td>
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<tr>
<td>2013</td>
<td>Asian PEDV in U.S.</td>
<td>2014</td>
<td>H10N8</td>
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<tr>
<td>2014</td>
<td>2nd strain of Asian PEDV, PRV</td>
<td>2014</td>
<td>Porcine delta-coronavirus</td>
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<td>2015</td>
<td>H5N8, H5N2, H5N1; canine H3N2</td>
<td>2015</td>
<td>Senecavirus A, pestivirus</td>
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<tr>
<td>2016</td>
<td>H7N8</td>
<td>2015</td>
<td>Japanese Encephalitis virus</td>
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