Agricultural Research Service FMD, CSF, ASF Programs

Secretary's Advisory
Committee On Animal Health
Washington DC
November 2, 2011

Luis L. Rodriguez DVM, Ph.D.
Research Leader, Agricultural Research Service,
Foreign Animal Disease Research Unit , Plum Island
Animal Disease Center

ARS Mission

ARS conducts research to develop and transfer solutions to agricultural problems of high national priority.

ARS @ Plum Island

Foreign Animal Disease Research Unit

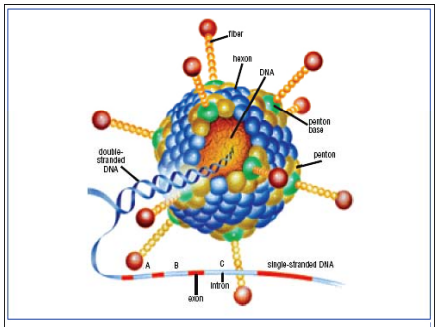
- Jonathan Arzt, DVM-Ph.D - Pathology
- Manuel Borca, DVM-Ph.D - Virology
- Teresa Delos Santos, Ph.D - Mol. Biology
- William Golde, Ph.D - Immunology
- Marvin Grubman, Ph.D - Virology
- Elizabeth Rieder, Ph.D – Mol Biology
- Luis L. Rodriguez, DVM-Ph.D - Virology
- James Zhu DVM-Ph.D - Bioinformatics

APPROVED NEW CRIS PROJECTS 2012-2017

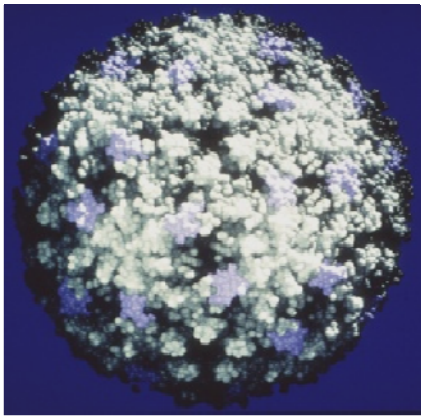
- Intervention Strategies to Support the Global Control and Eradication of FMD
- Countermeasures to Control Foreign Animal Diseases of Swine: CSF/ASF
- Ecology and pathogenesis of Re-Emerging VSV in North America

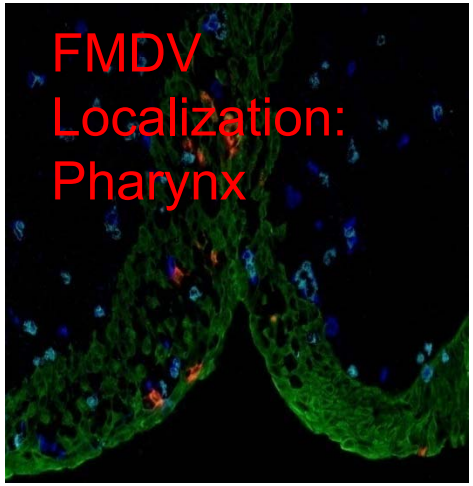
Recent Deliverables

- Real-time PCR tests for all major FADs
- Effective molecular FMD vaccine
- Marker CSF vaccine
- Discovery of primary site of FMDV replication
- New generation inactivated FMD vaccine
- Biotherapeutics that can protect swine against FMD in 24h

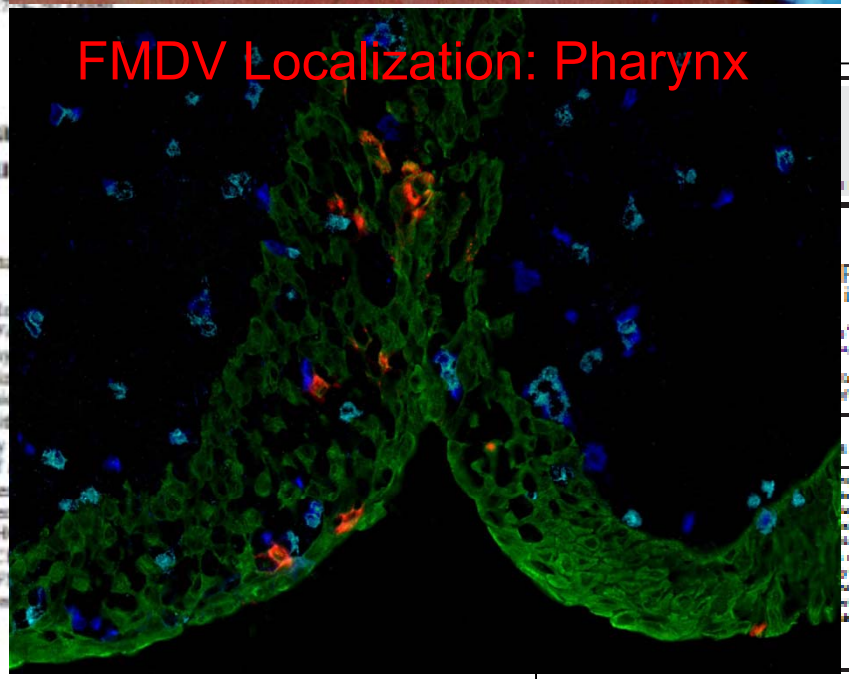


Ad5-FMD vaccine





FMD Pathogenesis 2010-2011



Early Pathogenesis of Foot-and-Mouth
ease in Cattle After Aerosol Inoculation:
tification of the Nasopharynx as the
nary Site of Infection

t[1,2], J. M. Pacheco[1], and L. L. Rodriguez[1]

Veterinary Pathology

January 2010 | Volume 47 | Number 1

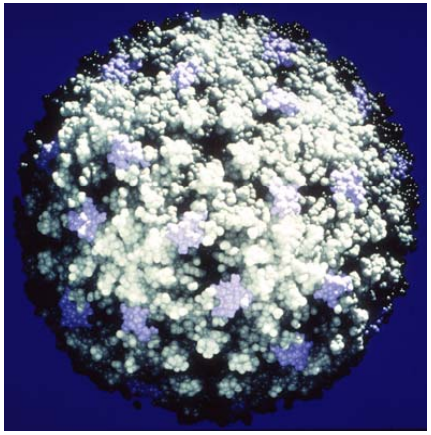
Veterinary Pathology

Special Focus:
Emerging Diseases and Global Surveillance

ACVP | ECVP | JCVP

Foot and Mouth Disease

- Small size, highly variable RNA virus (Picornavirus)
- *Closest relatives: bovine rhinitis virus*
- *Highly transmissible*
- *Seven serotypes: A, O, C, Asia, Sat1, Sat2, Sat3*



25 nm

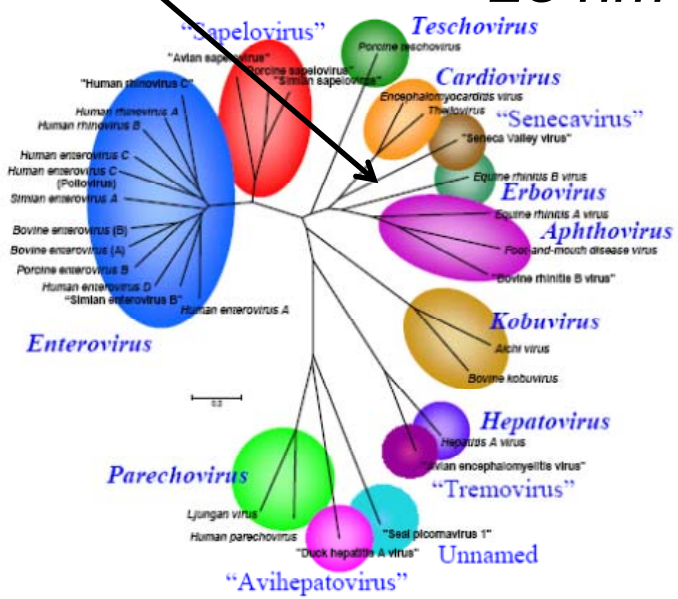
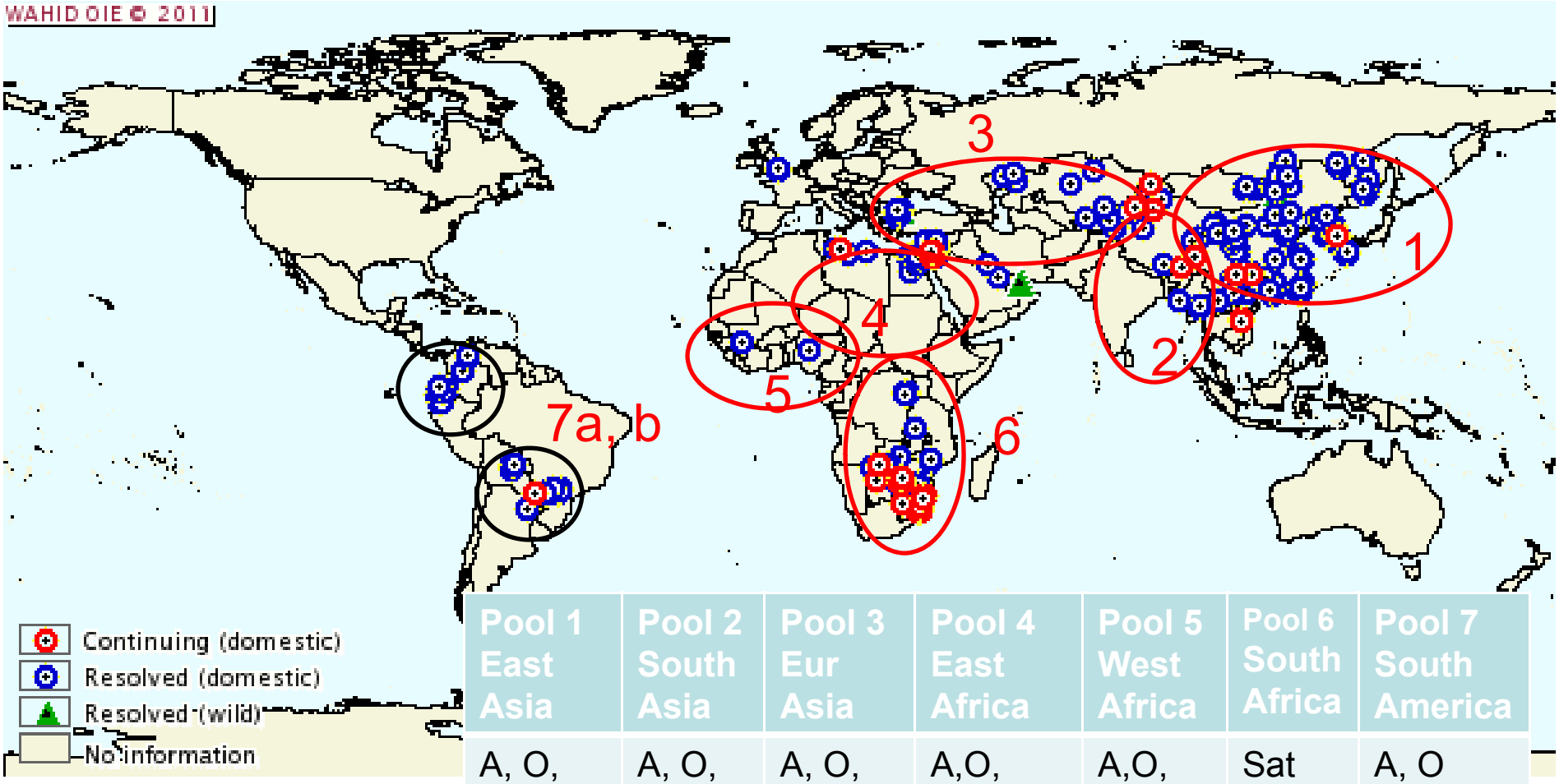


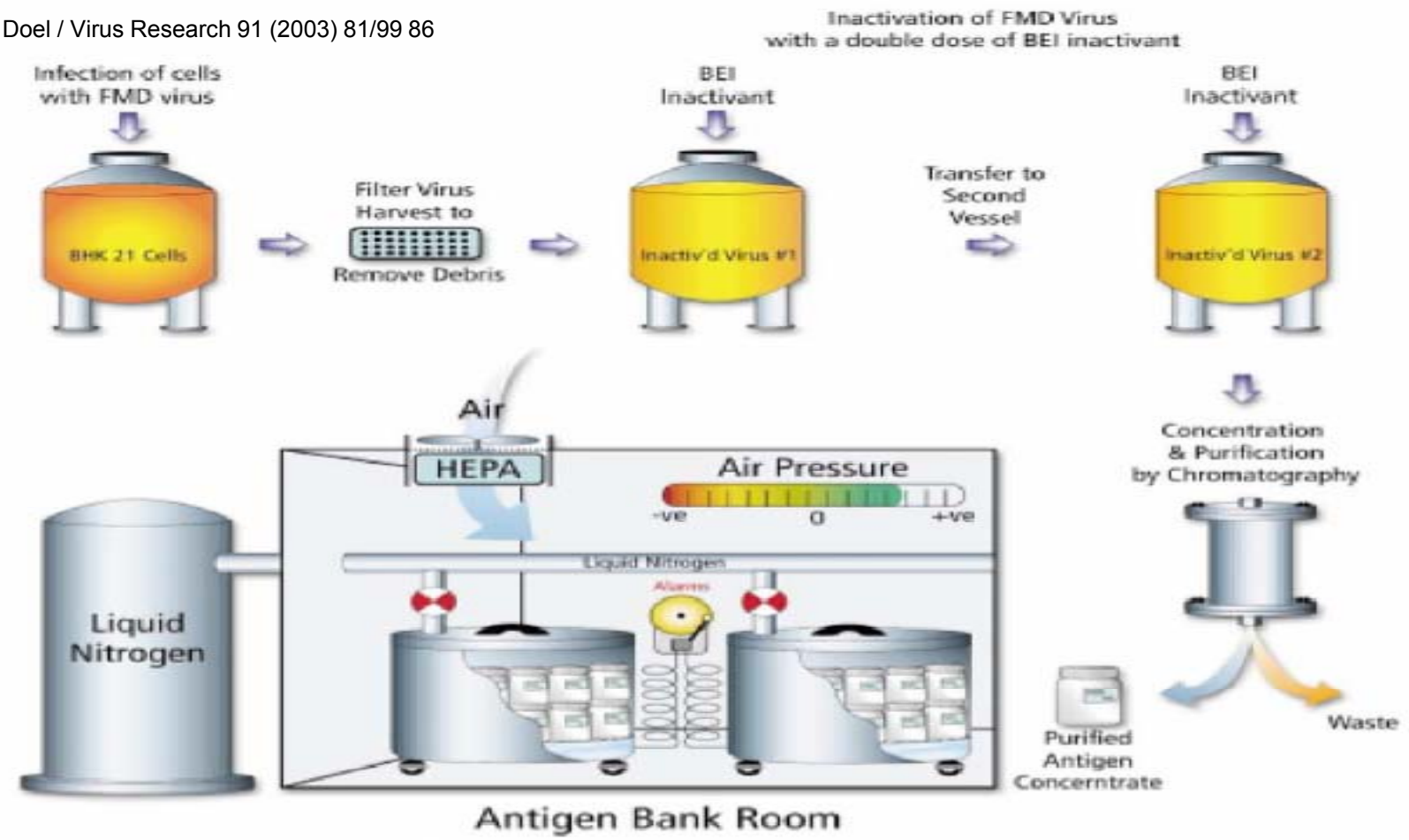
Figure 1. Unrooted Neighbor-joining tree of the *Picornaviridae* based on a comparison of the P1 capsid region.

FMD Outbreaks Reports 2005- 2011: Endemic Pools



Pool 1 East Asia	Pool 2 South Asia	Pool 3 Eur Asia	Pool 4 East Africa	Pool 5 West Africa	Pool 6 South Africa	Pool 7 South America
A, O, Asia 1	A, O, Asia 1	A, O, Asia 1	A,O, Sat 1-3	A,O, Sat1-2	Sat 1,2,3	A, O

T.R. Doel / Virus Research 91 (2003) 81/99 86



Inactivated Vaccine

Concerns with FMD Vaccines in *Disease-Free* Countries

- Require adaptation and growth of large volumes of wild type virus in cells
- Escape of virus from manufacturing facilities
- Require banking of multiple antigen concentrates
- Some antigens lack stability (low potency/short shelf life)
- Onset of protection 7-14 days
- Short duration of immunity ≤6 months
- Difficult to differentiate vaccinated from infected animals (DIVA) due to presence of NS proteins
- Vaccinated and exposed animals become carriers

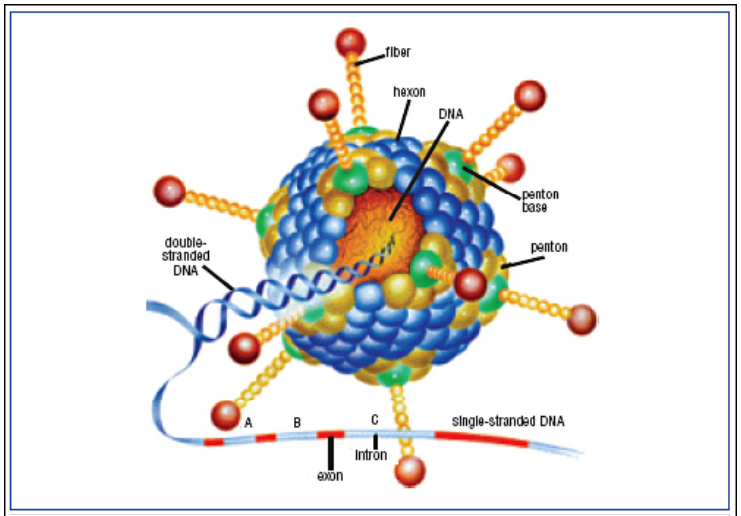
Characteristics of an "Ideal" FMD Vaccine

- Effective, rapid and **long-lasting** protection with one inoculation
- Prevents viral transmission
- Allow differentiation of infected from vaccinated animals (DIVA)
- Produced without the need for virulent FMDV
- Prevent development of carrier state
- Protection against multiple serotypes
- Stable antigen – long shelf life

Novel Subunit Vaccines

- A novel FMD vaccine was developed by ARS scientists under the leadership of Dr. Marvin Grubman

- This vaccine utilizes a defective human adenovirus vector to deliver genes coding for FMDV structural proteins

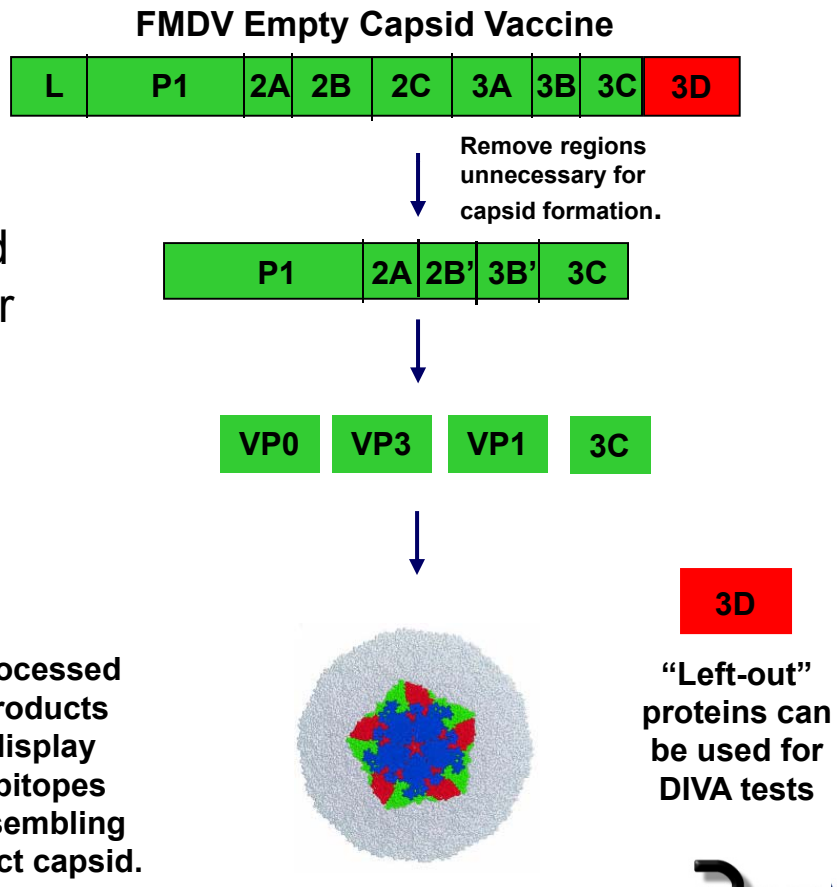


Human Defective Adenovirus 5 vector
- Lacks necessary proteins for growth
- Delivers and expresses transgenes in target cells

M. Grubman

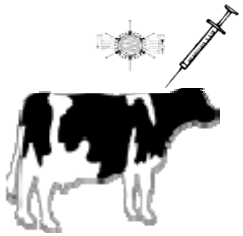
Multi-epitope Immunogen: Empty Viral Capsids (EVC or VLP)

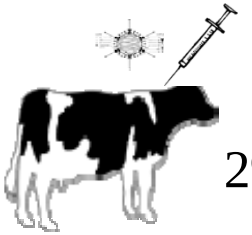
- Contains all protective epitopes present on current inactivated virus vaccine but lacks infectious viral nucleic acid and non-structural protein (NSP)
- Allows to *"cleanly"* distinguish vaccinated from infected animals using 3D and other NSP diagnostic tests
- Can be safely produced in the United States



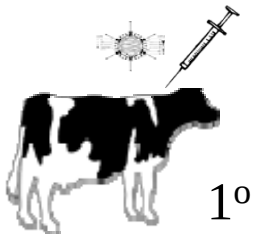
Currently under advanced development by DHS
Field test in US cattle – 3 States – 400 animals

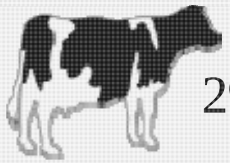
Ad5-FMD Vaccine Prevents Clinical Disease and Viral Transmission

N=6  1º Vx

N=4  2º Vx

No clinical disease 100% protection (10/10)

N=6  1º Vx

N=4  2º Naive

No clinical disease 100% protection (10/10)

N=6 1º Naive

N=4 2º Naive

100 % clinical disease (10/10)

Homeland Security

DHS- TAD Group

USDA

FMD Vaccine Product Profiles: Current Inactivated vs. Molecular

PRODUCT PROFILE	CURRENT INACTIVATED	Ad5-FMD
Prevents viral transmission	✓	✓
Early onset of immunity	✓	✓
Marked vaccine (DIVA capable)	+/-	✓
Domestic production (USA)	No	✓
Long-term stability formulated product	No	✓
Readily deployable (ready to use)	No	✓
Compatible with “vaccinate to live” strategy	No	✓
Provides cross-serotype protection	No	No

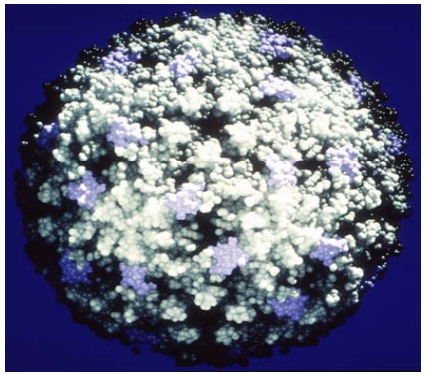
R&D Challenges Remaining For Ad5-FMD Vaccines

- **Potency(*) (Cost)**
- Duration of immunity(?)
- Prevention of carrier state
- Cross serotype protection

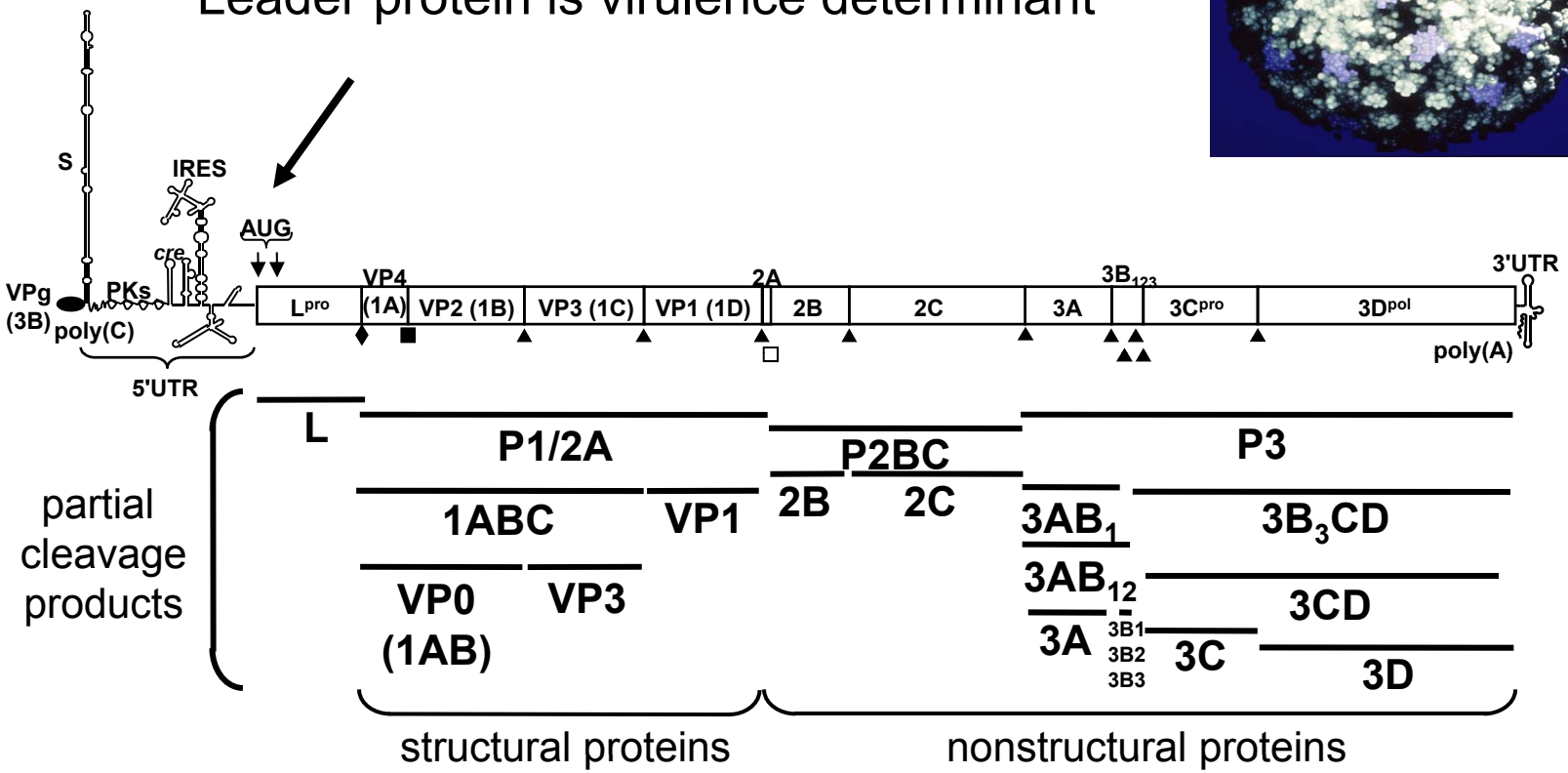
Future improvements of Ad5-FMD

Improvement	Problem addressed
Intradermal delivery	Potency
Multiple site delivery	Potency, duration of immunity?
Addition of adjuvants (IFN)	Potency, onset of immunity
Targeting specific tissues	Potency, carrier state?

Rationally Designed FMD Vaccines



Leader protein is virulence determinant



- Identification of genomic regions determining virulence
- Identification of antigenic epitopes associated to infection
- Engineering FMDV **to attenuate** and remove antigenic sites

Protease Cleavage Sites

L^{pro} ◆	unknown ■
2A □	$3C^{pro}$ ▲

FMDV Containing Mutations in Leader Become Attenuated In

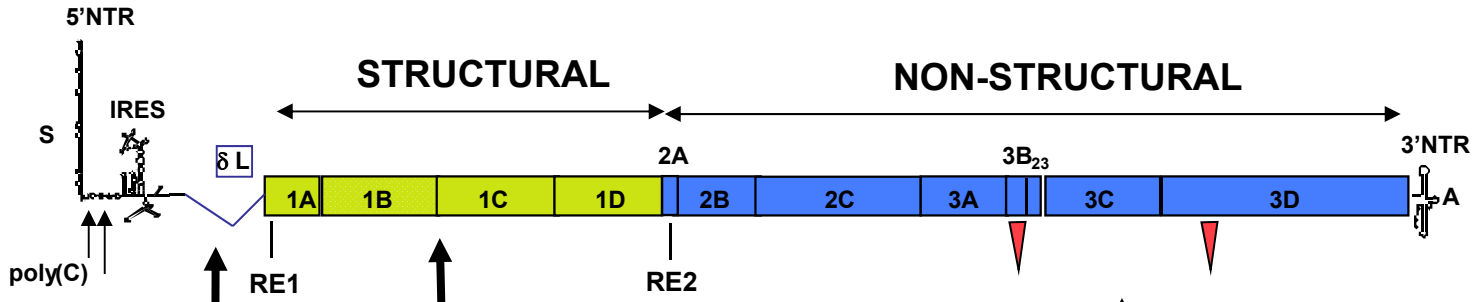
6-24h 24-48h 72-96h 96-240h >28 days?

Low titer

High titer

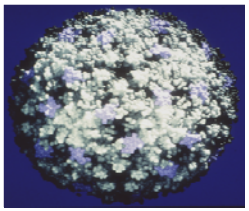
Possible approach for LAV?

Double marker cDNA-derived Killed FMDV Vaccine Platform



Negative markers Targeting NSP

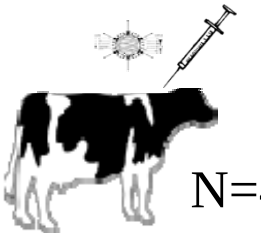
Easy swap of capsid sequences



Attenuating factor

Efficacy Data Cattle

BEI-inactivated Vaccine formulated with montadine ISA 260 adjuvant (Sepic-WOW)

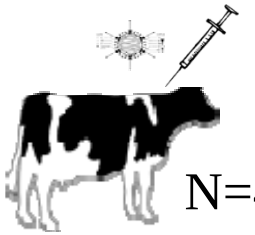


N=4

Commercial Tetravalent FMDV Vaccine

1xBEI- Vx

No clinical disease (0/4)*

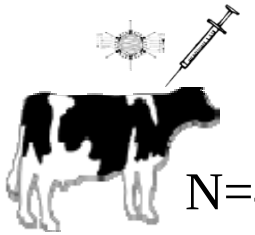


N=4

FMD-LL3D FMDV Vaccine

1^{x} BEI Marker virus

No clinical disease (0/4)*



N=4

FMD-LL3B3D FMDV Vaccine

1^{x} BEI Marker virus

No clinical disease (0/4)*

N=4

Naïve

unvaccinated controls

100 % clinical disease (4/4)

Transition status:

- Provisional patent 2010
- CRADA industry partner – development plan

Challenges For FMD Vaccines

- Understand the barrier of serotype- and subtype-specific vaccine protection (achieving cross-protection and/or increasing the breadth of antigenic coverage)
- Improve the onset and duration of immunity of current and next generation FMD vaccines
- Target FMD vaccines to induce protection at relevant tissues to prevent infection (hence also prevent persistence)

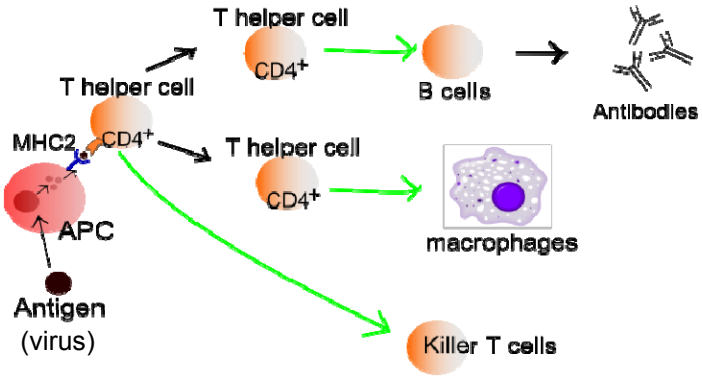
Analysis of T cell Responses to Livestock Vaccines

Classical Swine Fever (CSF)

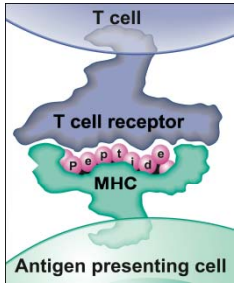
African Swine Fever (ASF)

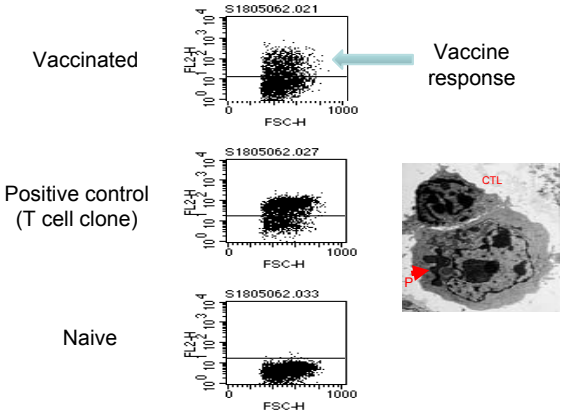
Foot-and-Mouth Disease (FMD)

Vaccine performance depends upon stimulating specific immune responses of T cells - but what do T cells recognize



21st century technology adapted to cattle and swine (MHC tetramers and sequence T cell receptors from vaccinated and infected animals)





Provides information on T-cell antigens

Allows analysis of *in vivo* responses

Rapid assessment of immune response to vaccination

Rapid and accurate go/no go decisions for vaccine formulations and delivery

USDA - Plum Island Animal Disease Center (W. Golde), University of Copenhagen (S. Buus), University of Vermont (J. Barlow), Washington State University (W. Brown)

Novel Strategies to Control FMD: The Use of Interferon

- **Previous work:**
- Swine administered Ad5-porcine interferon alpha are protected 1 day later when challenged with FMDV A, O or Asia-1
- Protection can last 3-5 days
- Combination Ad5-FMD vaccine and Ad5-pIFN can induce rapid and long-lasting protection
- Similar approach in cattle – not successful so far

A Novel Effective Interferon Identified Through Bioinformatics

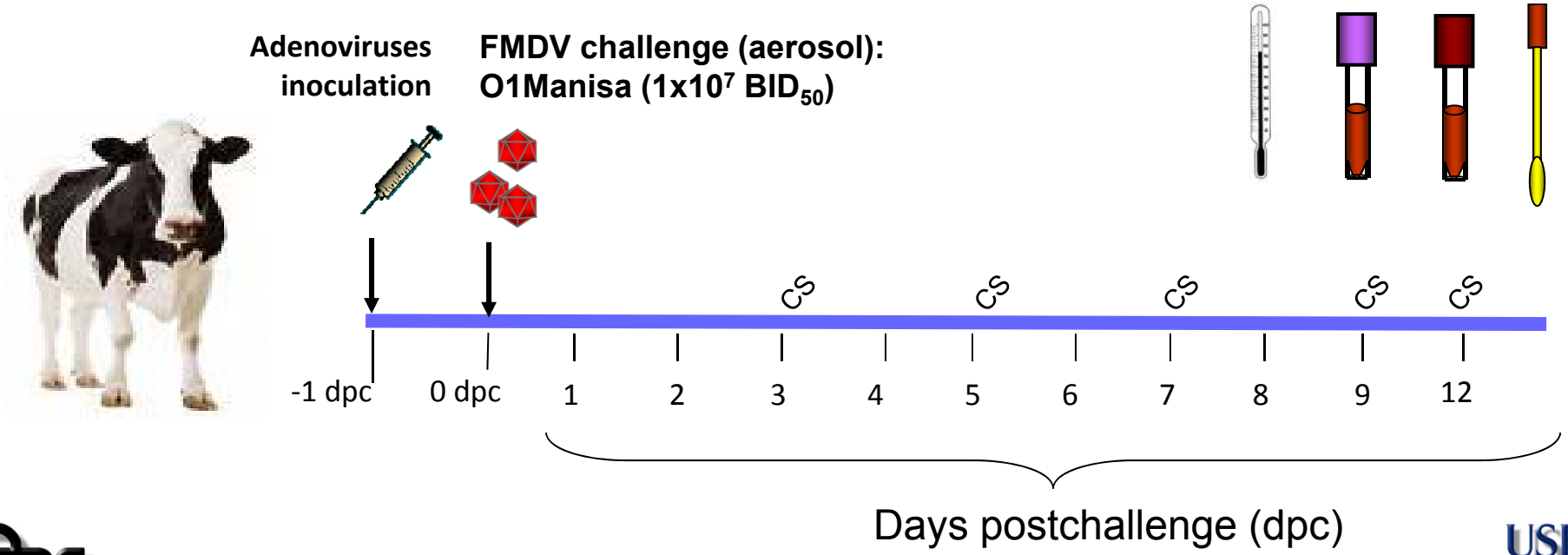
Inteferon Lambda Treatment (3 animals/group)

I. PBS

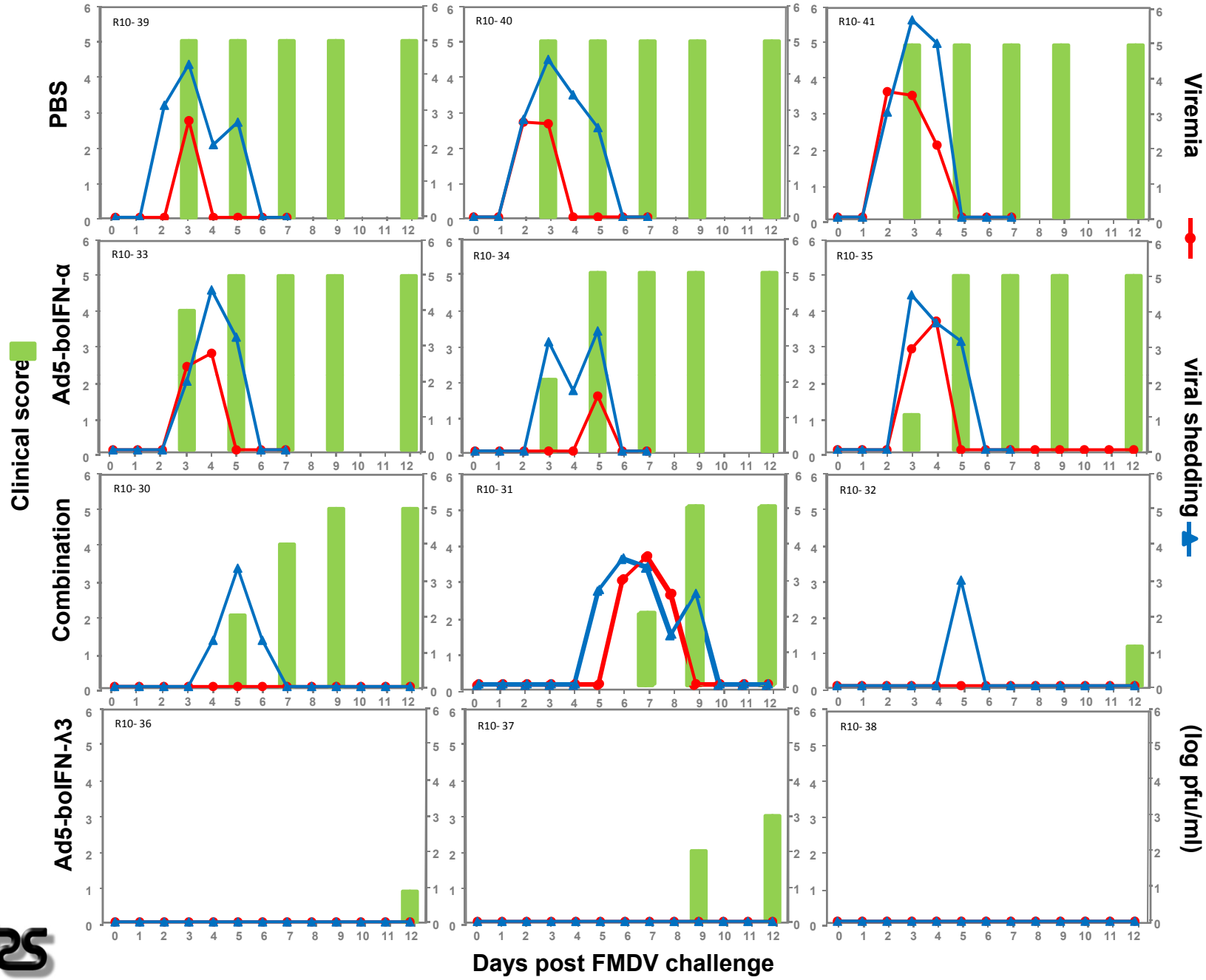
II. Ad5-boIFN-α (1.5x10^{11} pfu)

III. Ad5-boIFN-λ3 (1.5x10^{11} pfu)

IV. Combination: Ad5-boIFN-α (0.75x10^{11} pfu) + Ad5-boIFN-λ3 (0.75x10^{11} pfu)



Clinical data: clinical signs, viremia and FMDV shedding



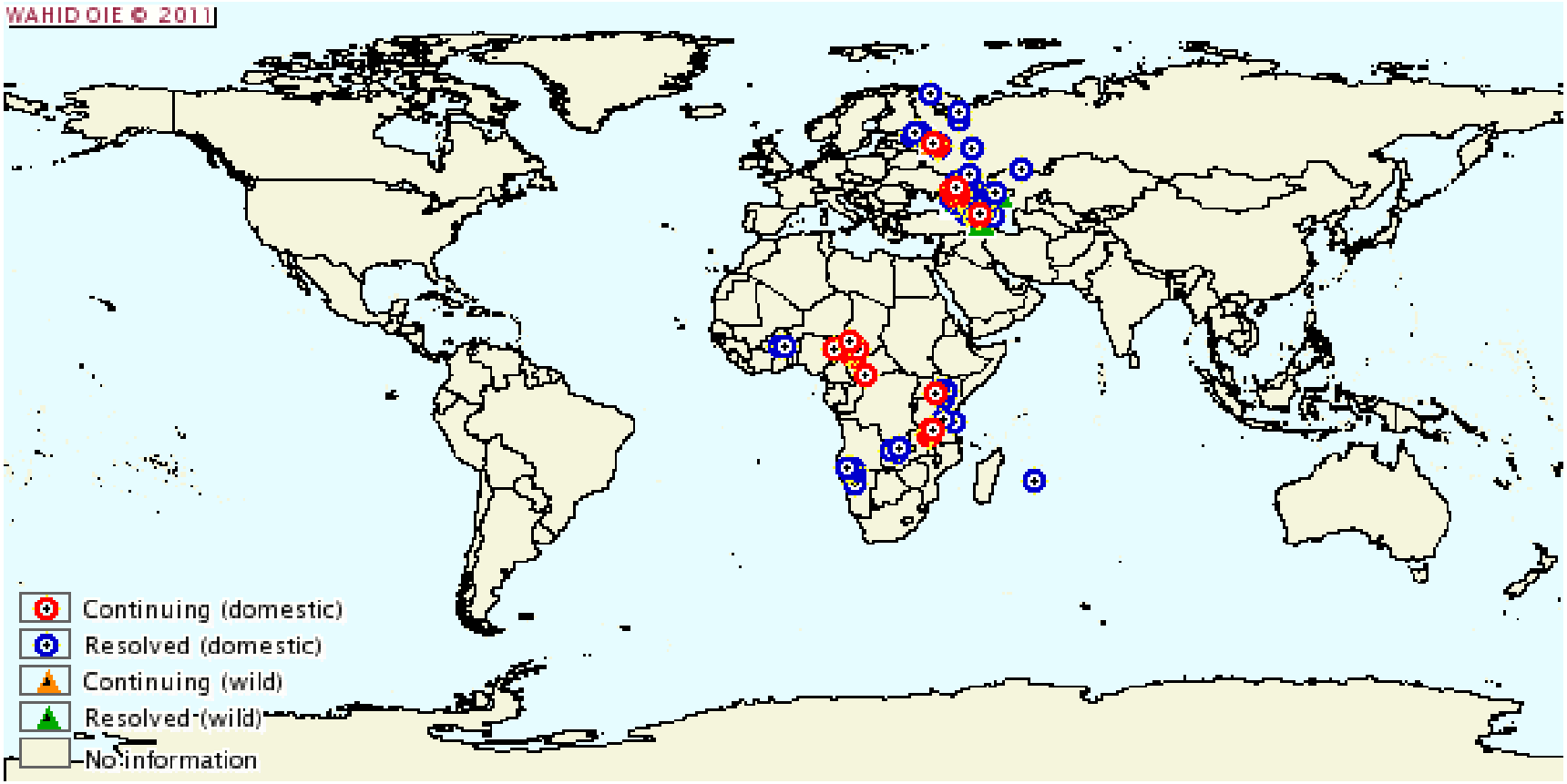
There is a need for vaccines that are inexpensive to produce, easy to deliver and induce long-term immunity. Also there is need for better integrated strategies that fit the specific needs of endemic regions. Only when these critical components are available will the global eradication of FMDV be possible

Veterinary research can improve the lives of millions of people around the globe!

African Swine Fever Virus

- High mortality (100%) in domestic swine
- Endemic in Africa
- Recent incursion in the Caucasus region
- Major threat to swine industry in US and Europe
- No vaccine available!

ASF On The Move 2011



Beginning of 2007 in R. of Georgia and has since spread to the neighboring countries of Armenia, Azerbaijan and Russia

Recent Headlines

Russia Discovers Seven Mor

www.bloomberg.com/news/2011-10-31/russia-discovers-seven-more-cases-of-african-swine-fever-in-pigs.html

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Russia Discovers Seven More Cases of African Swine Fever in Pigs

By Marina Sysoyeva - Oct 31, 2011 2:46 AM ET

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Russia discovered seven more cases of African swine fever in pigs.

Five were found in the Krasnodar region, one in the Bagatugtunskoye village in the Republic of Kalmykia, and one in the town of Kursk in central Russia, the government's Rosselkhoznadzor said today in separate e-mailed statements. Krasnodar and Kalmykia are in southern Russia.

The virus killed 20,000 pigs in southern Russia in the first half of the year, according to Rosselkhoznadzor data.

To contact the reporter on this story: Marina Sysoyeva in Moscow msysoyeva@bloomberg.net

To contact the editor responsible for this story: Claudia Carpenter at ccarpenter2@bloomberg.net

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Russia to launch new offensive against ASF

Tuesday November 01 2011

Russia is preparing to announce new measures aimed at combating African Swine Fever (ASF) after confirming the pig disease has spread to previously unaffected parts of the country.

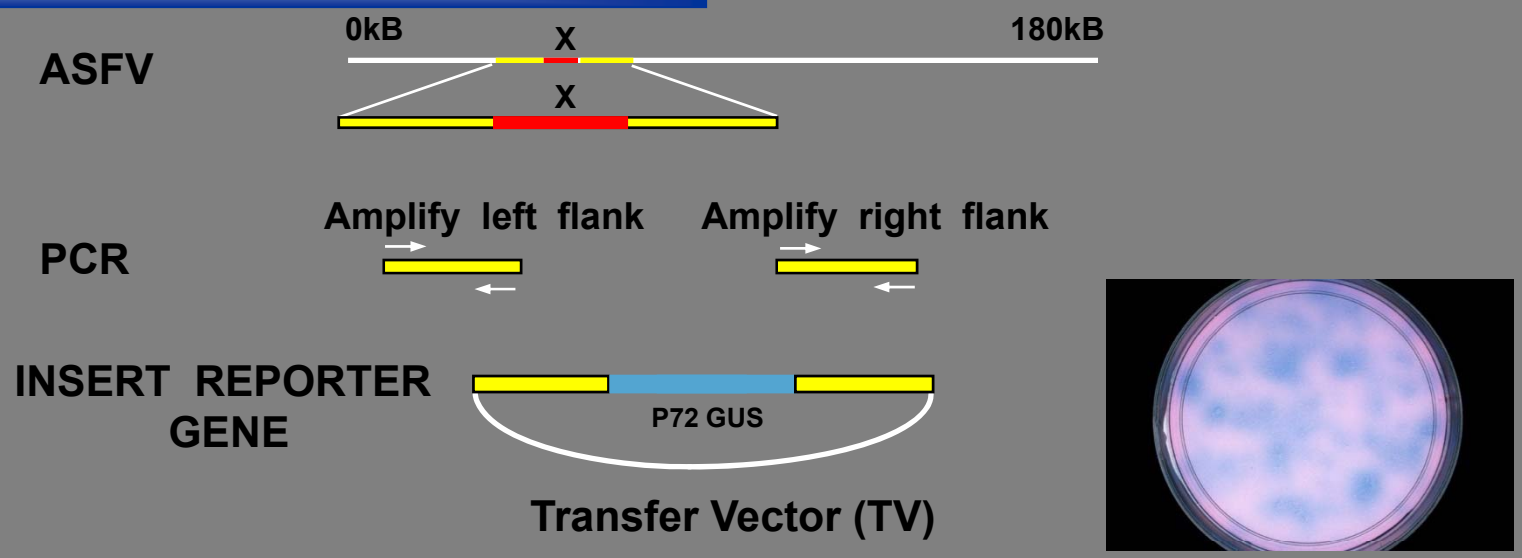
The federal veterinary service Rosselkhoznadzor said on Monday seven more cases of the virus had been detected at various locations - including one in Kursk, in Central Russia.

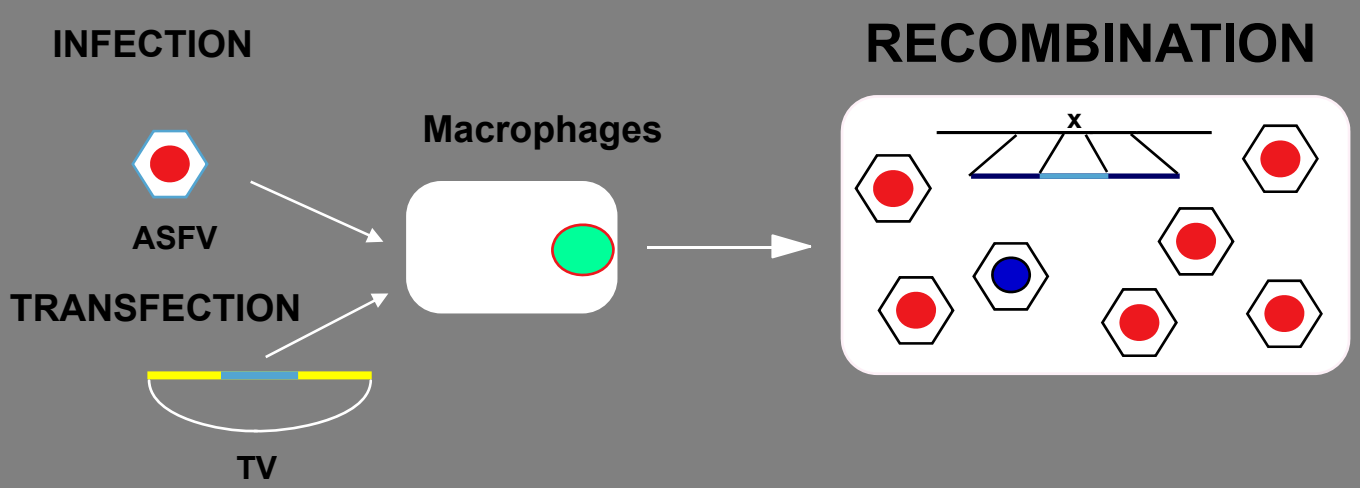
http://www.agra-net.com/portal2/home.jsp?template=newsarticle&artid=20017915336&pubid=ag002

ASF RESEARCH GAPS

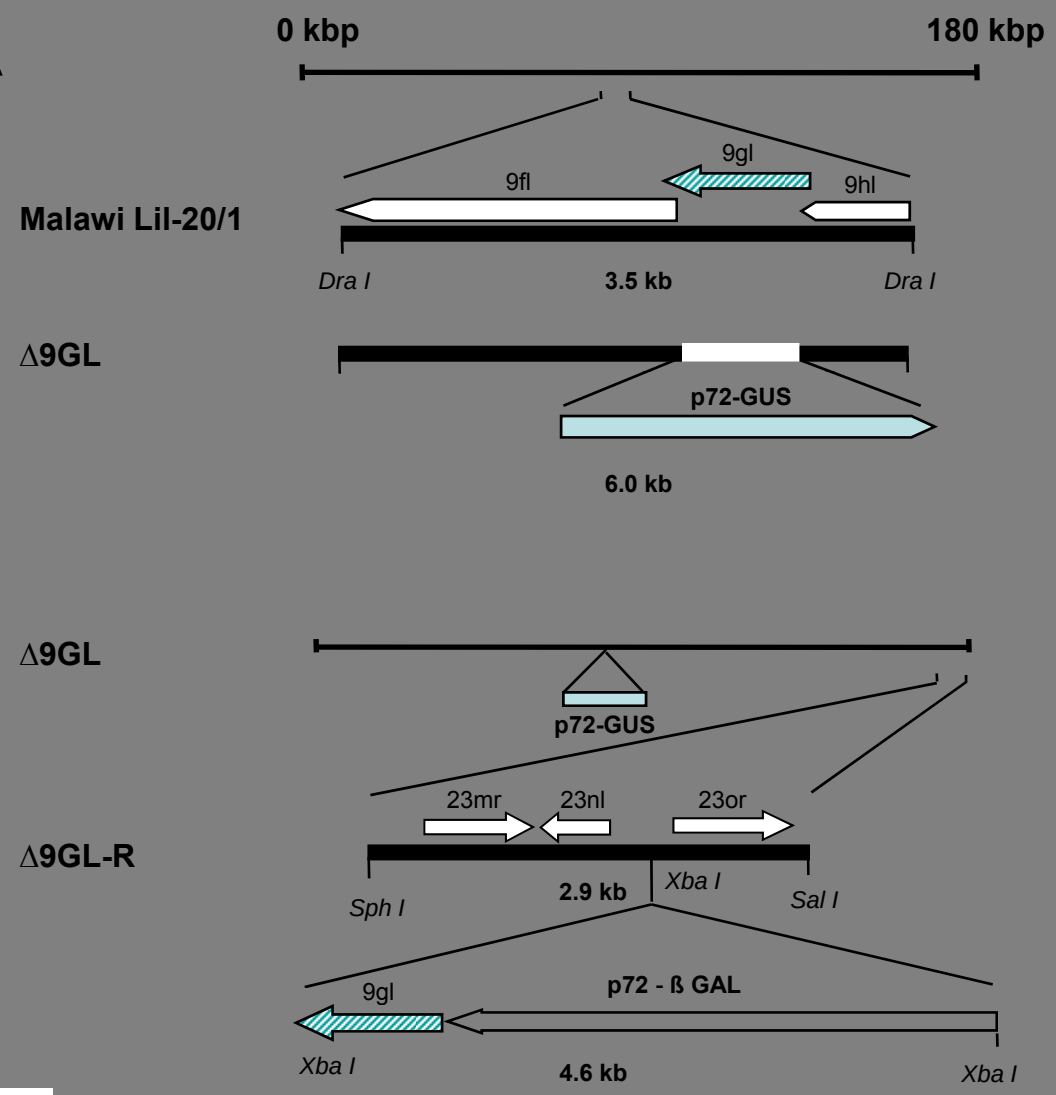
- ***Pathogenesis:*** *viral and host virulence determinants – early events in infection*
- ***Virus ecology:*** *host tick - virus interactions – role in transmission*
- ***Immunology:*** *protective immune response unknown there are no effective vaccines!*
- ***Epidemiology:*** *transmission cycles – direct vs vector*

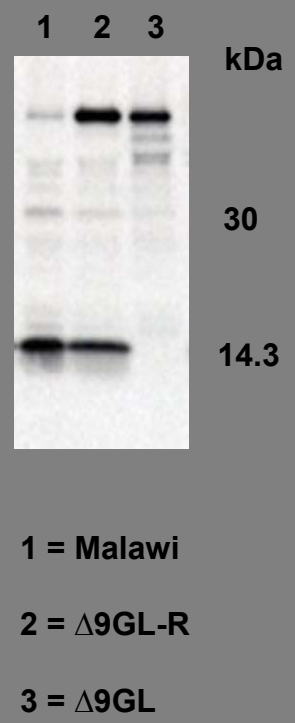
ARS Developed Only Successful Reverse Genetics System for ASF





Construction of Δ9GL and 9GL-R recombinant viruses

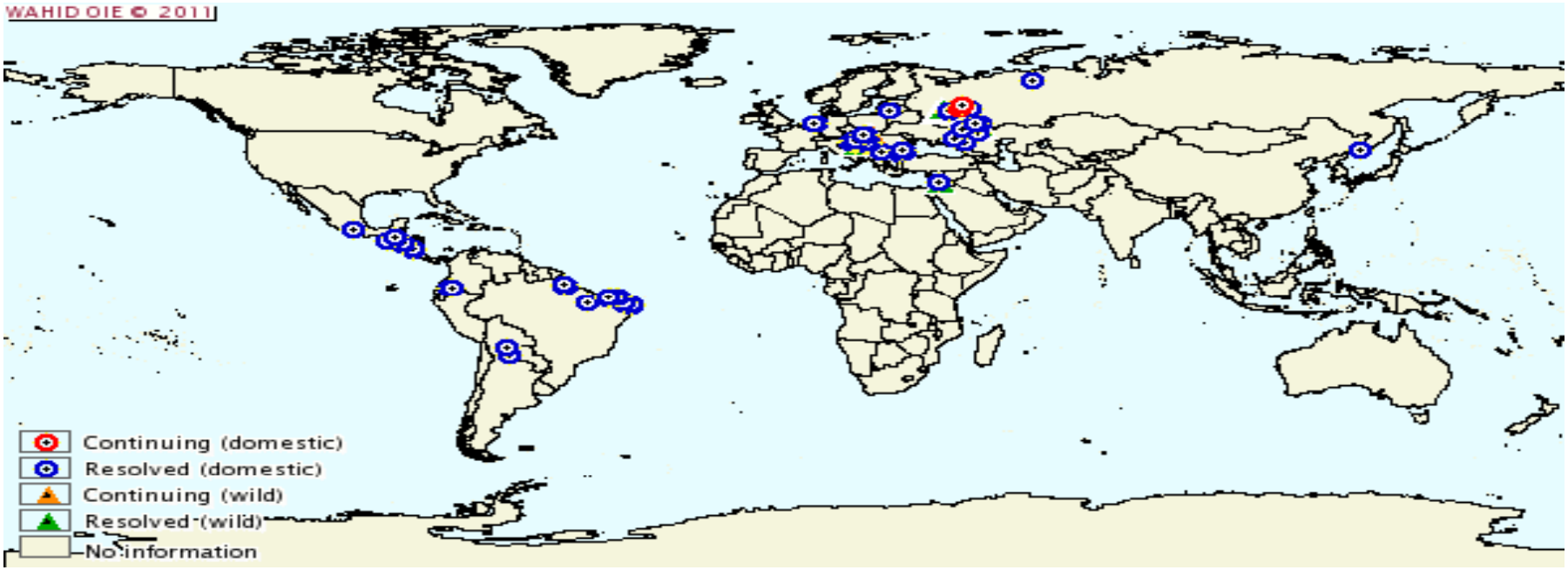


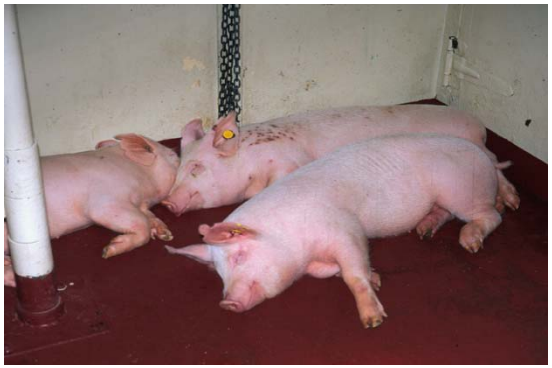


Δ9GL confers strain-specific protection

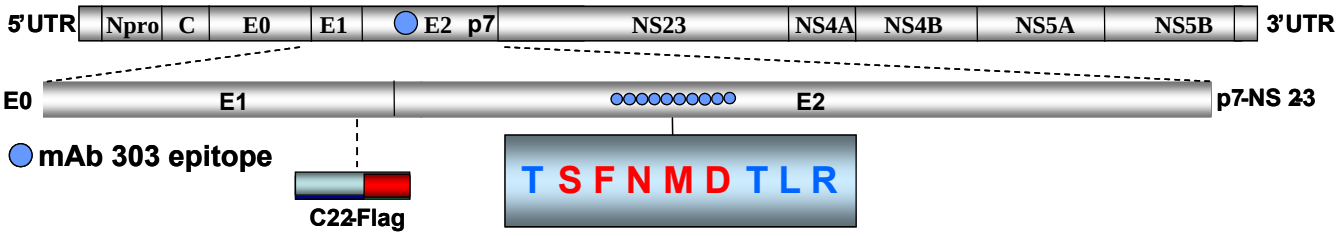
ASFV challenge (1,000-10,000 LD_{100})	Immunization: MALΔ9GL	TENΔ9GL	Pr4Δ9GL
	Protection in pig		
MAL (Malawi'83)	YES	NO	NO
TEN (Tengani'54)	NO	YES	NO
RSA1 (Pr4'96)	NO	NO	YES
RSA2 (Pr5'96)			YES
RSA3 (CR1'96)			YES
RSA4 (CR3'96)			YES
RSA5 (O1'96)			YES

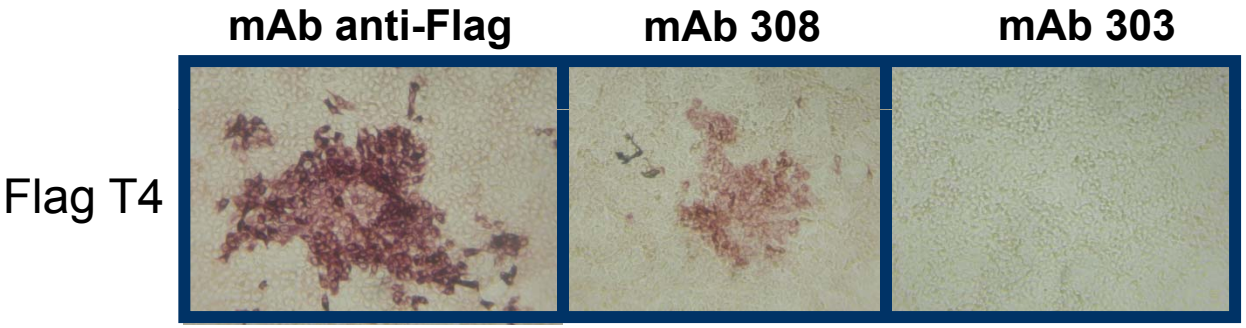
Classical Swine Fever Virus





Flag T4 virus as vaccine candidate





Vaccine / Challenge time	No. of survivors / total	CSF Symptoms / time of death days (+/-SD)	Mean time of fever onset days (+/-SD)	Mean time duration of fever days (+/- SD)	Max daily temperature average days (+/- SD)
Flag-T4v / 0 dpv	0/4	Yes / 10 (1.2)	4 (0.8)	5.2 (2.1)	105.7 (0.8)
Flag-T4v / 1 dpv	1/4	Yes / 13.6 (1.5)	6.2 (2.5)	5 (3.7)	106 (0.5)
Flag-T4v / 2 dpv	4/4	No	No	No	103.2 (0.7)
Flag-T4v / 3 dpv	4/4	No	No	No	103.2 (0.9)
Flag-T4v / 28 dpv	4/4	No	No	No	102.2 (0.4)
Mock vaccinated	0/4	Yes / 9.5 (0.7)	4.5 (0.7)	5 (1.4)	105.5 (1.2)

Determination of FlagT4v Minimal Protective Dose: Protection against the challenge

Vaccine Treatment	No. of survivors/total no.[h]	Mean time to death (days ± SD)	Fever	
			No. of days to onset (days ± SD)	Duration no. of days (days ± SD)
FlagT4v 10^5	9/9[a]	-	-	-
FlagT4v 10^4	8/10[b]	12 (4.2)[c][f]	6 (0.0)[f]	6 (4.2)[f]
FlagT4v 10^3	8/10[d]	10.5 (3.5)[e][f]	4 (1.4)[f]	7 (5.6)[f]
Mock	0/10[g]	9.3 (1.6)	3.8 (0.8)	5.5 (1.5)

Transition status:

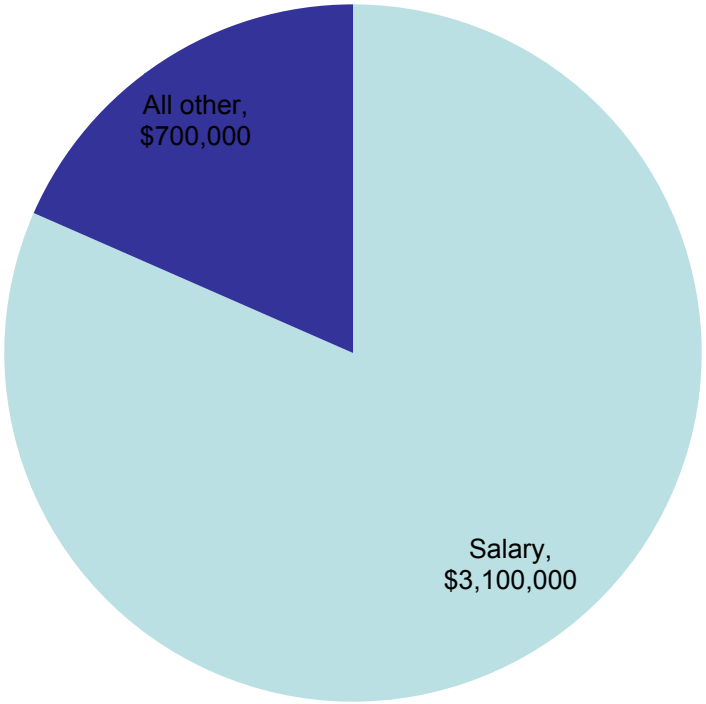
•Vaccine is currently under development in partnership with private industry

ARS Strategy For Countermeasure Development

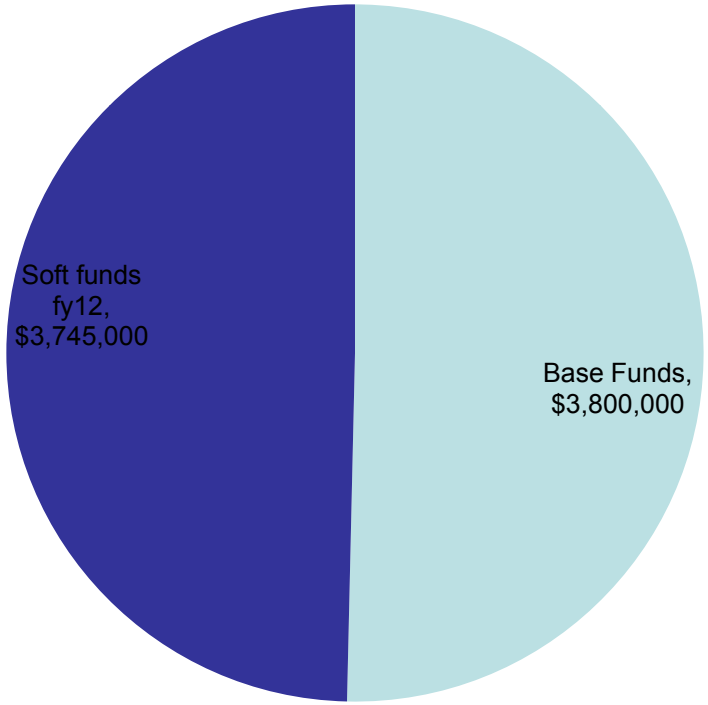
- Leverage ARS science with other agencies (DHS, APHIS)
- Engage Industry partners early in development
- Engage regulatory authorities early (CVB)
- Engage APHIS – NVS : provide preview of countermeasure pipeline

FY11 Funding

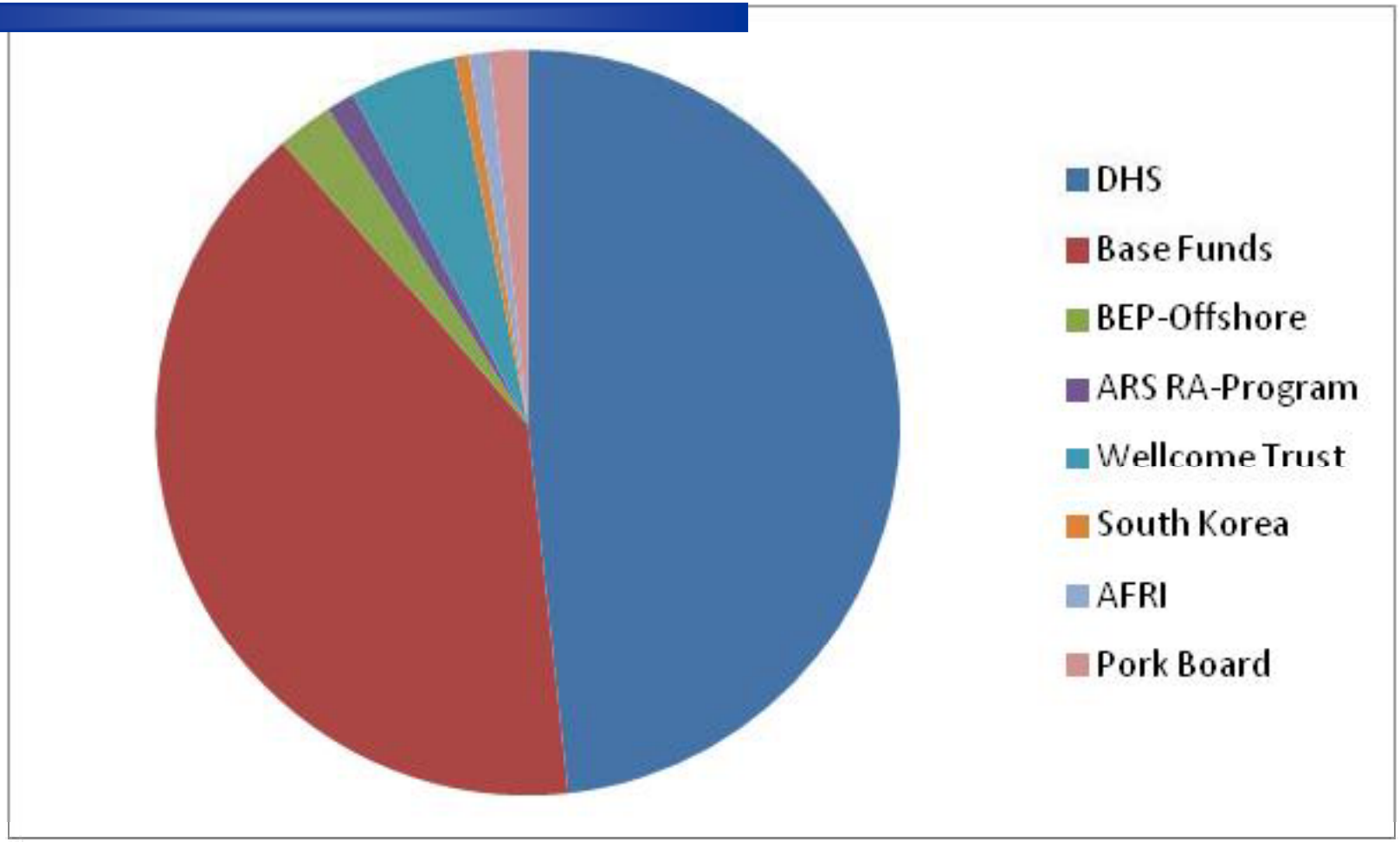
USDA-ARS funding



USDA + Soft funds



Funding FY12 (??)



Total FY12 ~$7M