ANIMAL VIRUSES USED FOR VACCINATION OF HUMANS

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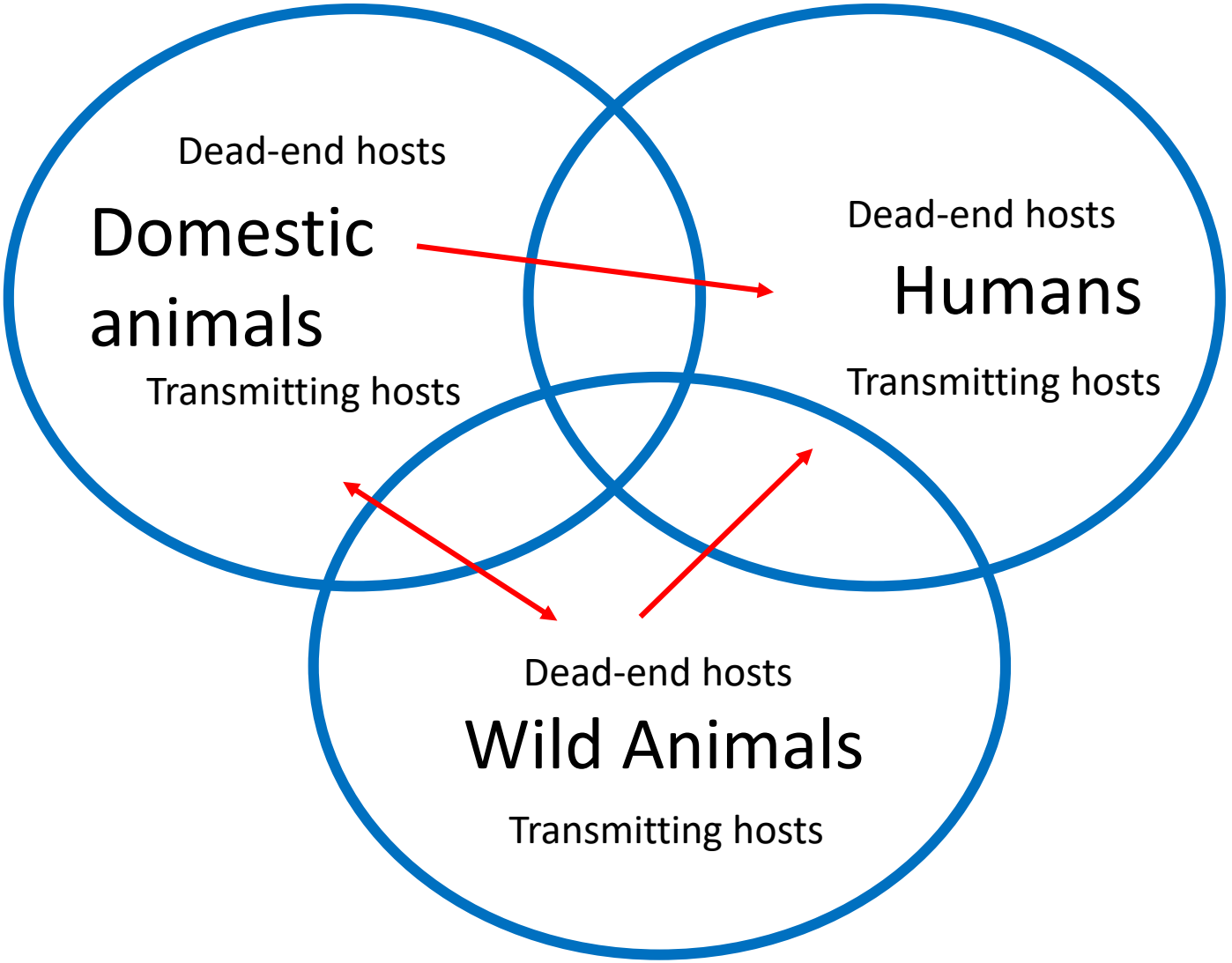
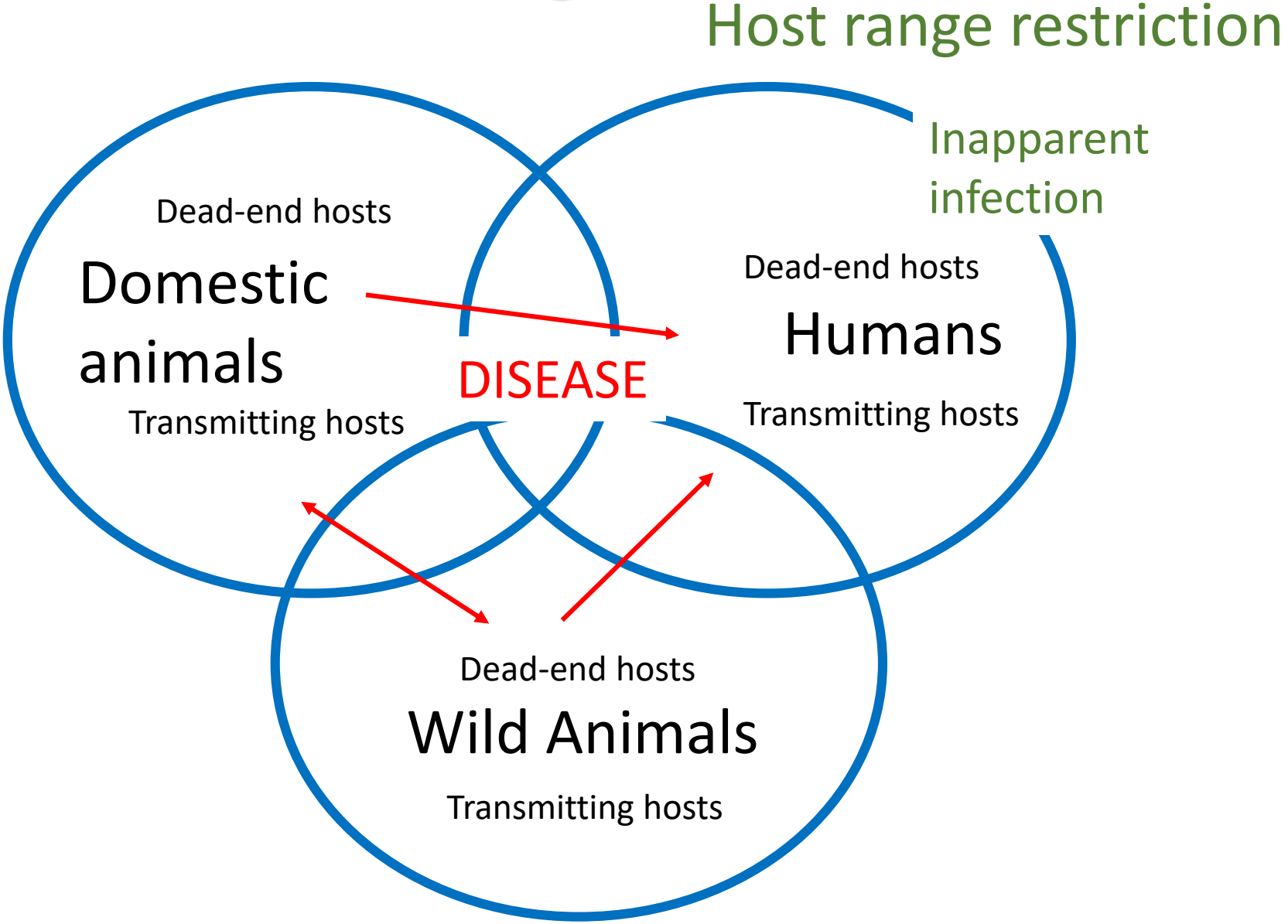
Disclosure

- Dr. Monath has current financial interests in
  - Crozet BioPharma LLC
  - US Biologic Inc
  - Public Health Vaccines LLC
- Previous financial interests in:
  - NewLink Genetics Corp

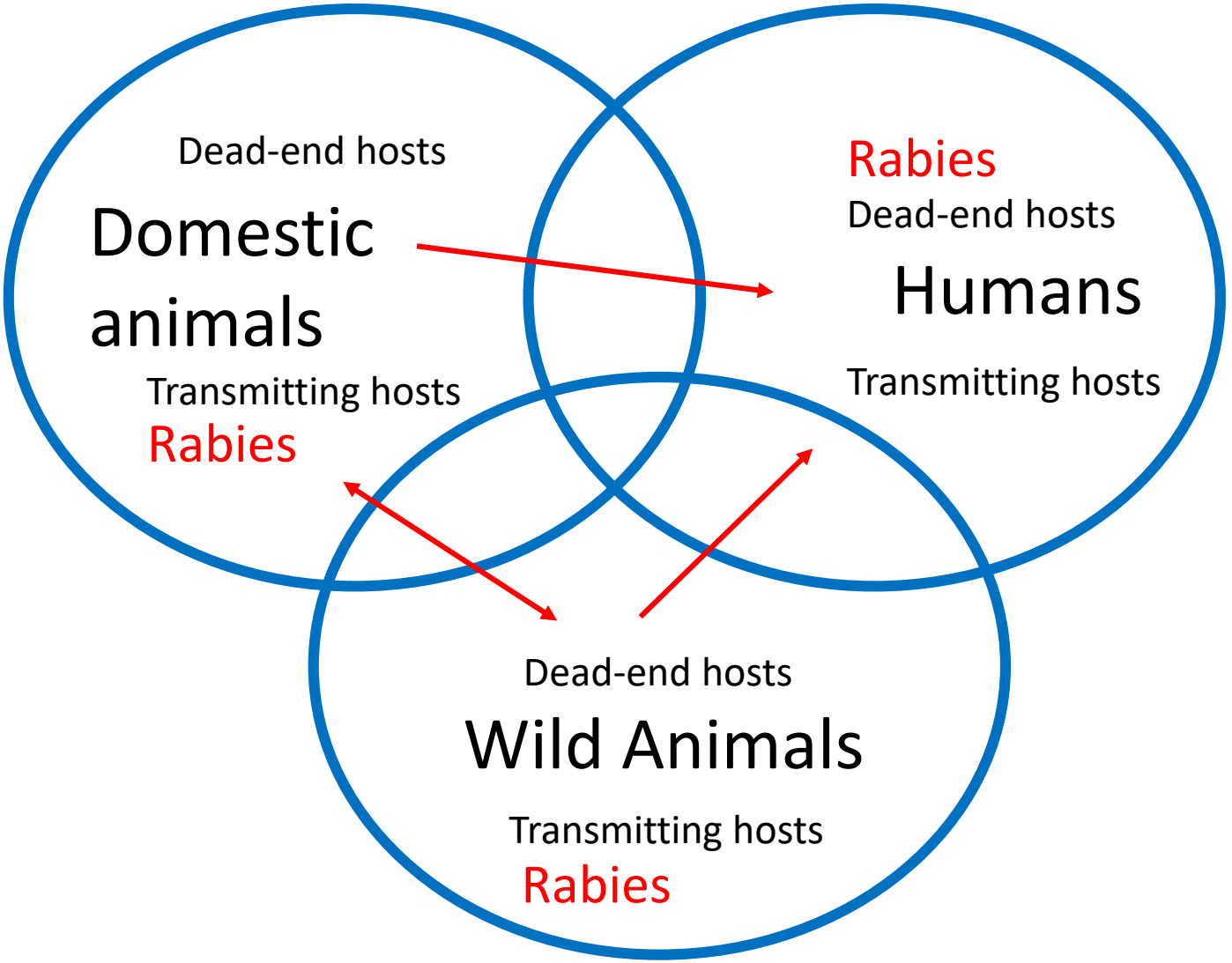
One Health Paradigm



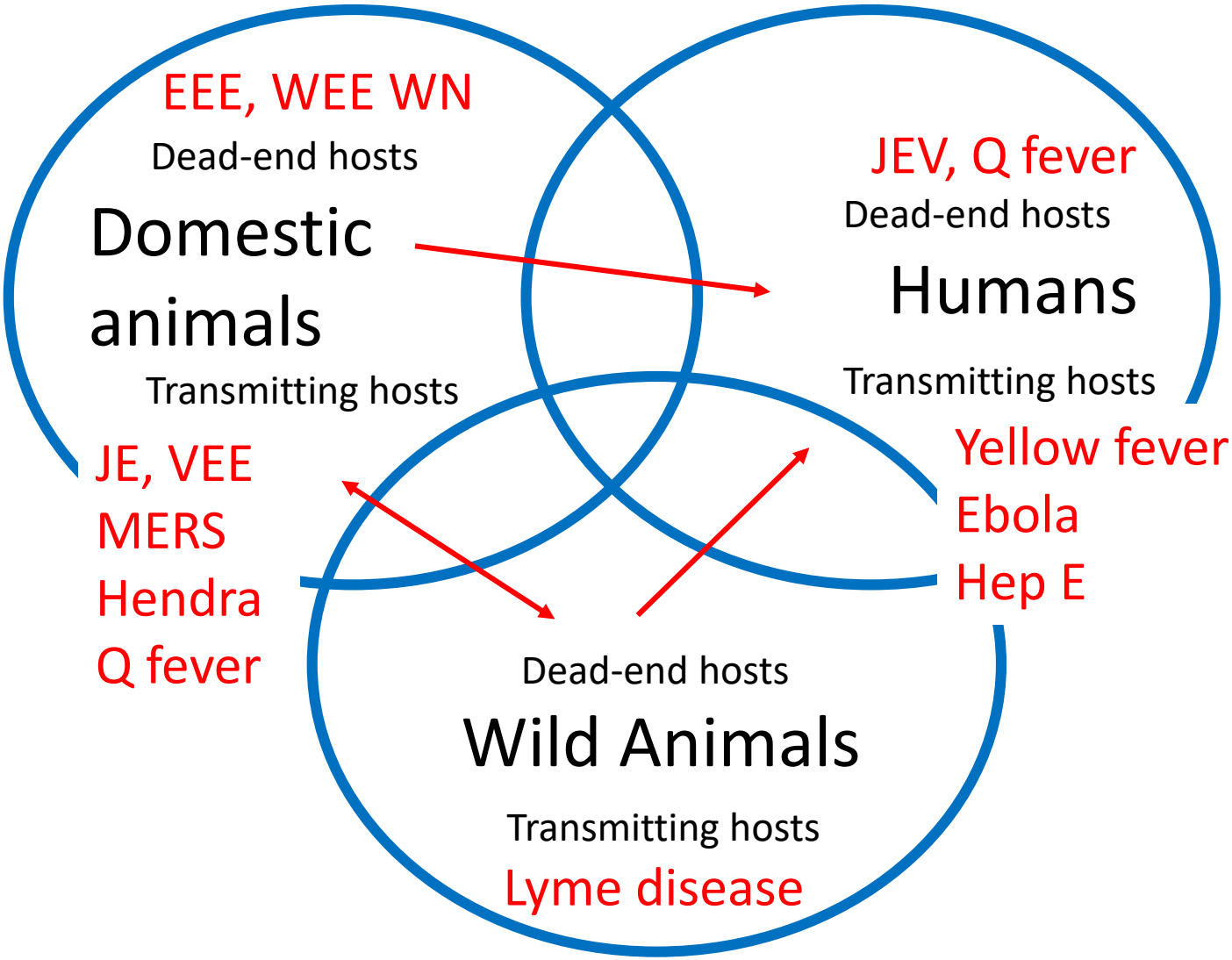
One Health Paradigm



Prevention and control of zoonotic infections by vaccination



Prevention and control of zoonotic infections by vaccination

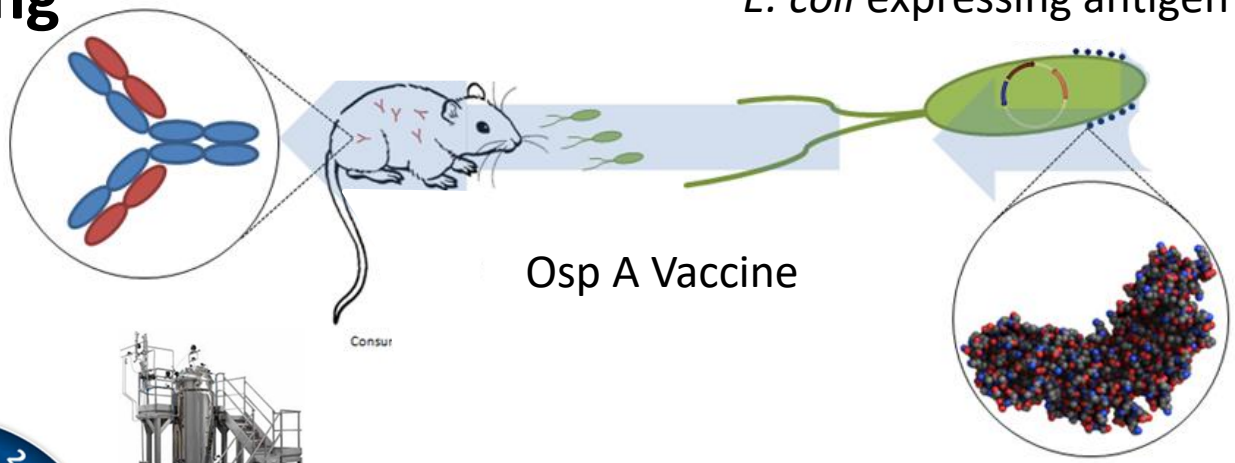


Oral vaccine that breaks the transmission cycle of the Lyme disease spirochete can be delivered via bait

Maria J.C. Gomes-Solecki [a,b,*], Dustin R. Brisson [c], Raymond J. Dattwyler [a,b]

Vaccine 24 (2006) 4440–4449

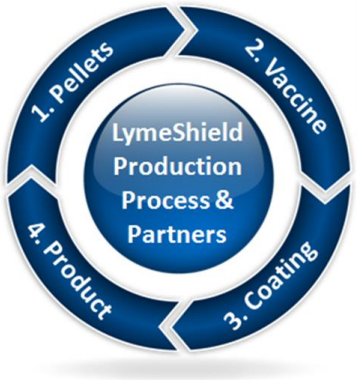
Reservior Targeting Vaccine Lyme Disease



E. coli expressing antigen

Osp A Vaccine

LabDiet



HENNESSY RESEARCH

US BIOLOGIC

US BIOLOGIC Agricenter International

Feeding station for *Peromyscus*

Animal viruses used in human vaccinology

- Animal viruses (bacteria) modified or inactivated to protect against the *homologous* disease agent of humans
  - Vaccines against the corresponding zoonosis
  - Many examples, e.g. anthrax, plague, Q fever, yellow fever, JEV, VEE, TBE, etc.
- Animal viruses (bacteria) used to protect against a *heterologous* disease agent of humans
  - Used directly
    - Naturally attenuated for humans due to restricted host range
    - Cross-protects against the human disease agent Jennerian vaccine)
  - Used as a vector of the gene/antigen of interest

Animal viruses (bacteria) used to protect against a *heterologous* disease agent of humans

- Naturally attenuated, host range restricted, used directly without modification (**true Jennerian vaccine**)

Animal agent	Type	Human indication
Mycobacterium microti	Live, host-range restricted	*M. tuberculosis*
Mycobacterium vaccae (M. obuense)	Inactivated, whole cell	*M. tuberculosis*
Vaccinia, MVA	Live, host range restricted	Variola
Simian, bovine, ovine rotaviruses	Live, host range restricted	Human rotaviruses
Sendai (mouse parainfluenza-1)	Live, host range restricted	hPIV1
Langat virus	Live, host range restricted	Tick-borne encephalitis

In red: approved or in registration

Animal viruses (bacteria) used to protect against a *heterologous* disease agent of humans

- Recombinant technology using animal viruses as a vector

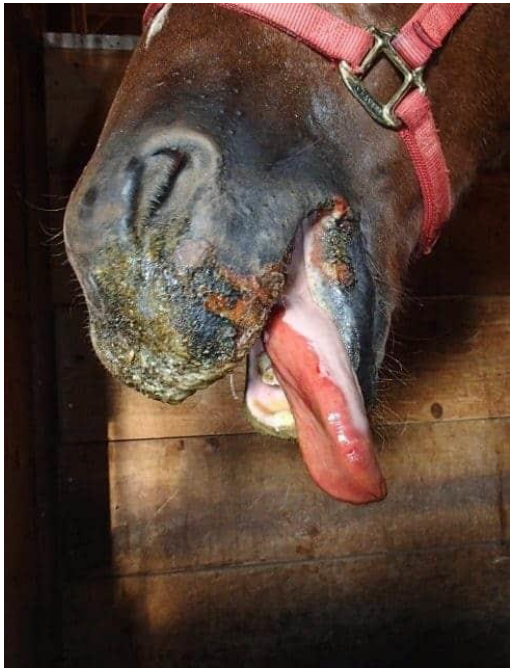
Animal agent	Type	Human indication
Mycobacterium bovis (BCG)	Live, recombinant	*M. tb* and multiple other targets
Bovine rotavirus	Live, bovine-human reassortants	Human rotavirus
Vaccinia, MVA, canarypox	Live, recombinant (replicating or defective)	Multiple targets
Chimpanzee adenoviruses (+/- MVA boost)	Live, recombinant (defective)	Ebola, Sudan, HIV, HCV, RSV, rabies
Vesicular stomatitis	Live, recombinant (replicating)	Ebola, Marburg, Lassa, Nipah, SARS
Bovine parainfluenza type 3	Live, recombinant (replicating)	RSV, hPIV1-4, hMPV, SARS
Sendai (mouse parainfluenza-1)	Live, recombinant (replicating)	RSV, hPIV1-4, hMPV, HIV
Canine distemper	Live, recombinant (replicating)	HIV, rabies
Newcastle disease virus	Live, recombinant (replicating)	RSV, Influenza, polio, SARS, Ebola
Flaviviruses (YF, dengue, Langat)	Live, recombinant (replicating or *defective*)	Dengue, JEV, West Nile (vet.), TBE, Zika, *flu, RSV, HIV, rabies, malaria*
Alphaviruses (VEE, CHIK, Sindbis, SFV)	Live, recombinant (replicating, *defective*)	EEE, WEE, RRV, *HIV, SARS, HSV, HPV*
Rabies	Recombinant, defective or inactivated	Marburg
LCMV	Recombinant, *defective*	CMV

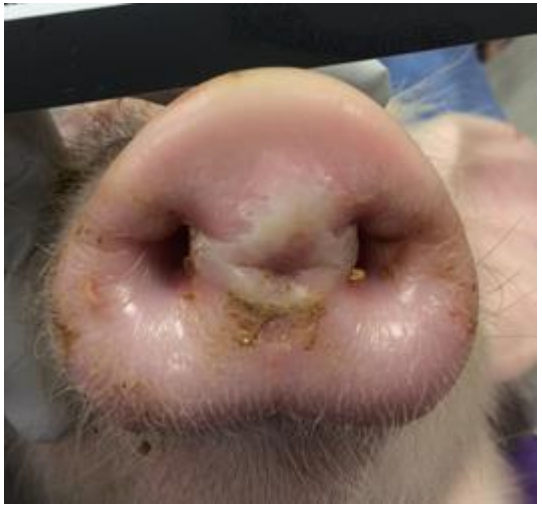
In red: approved or in registration

Shaded: In clinical development, some with promising results

Advantages of recombinant animal viruses used as vaccines

- Platform technology for multiple indications
- Attenuated by host restriction or defective, single cycle
- Replicating constructs generally provide rapid protection after a single dose
- Memory responses, durable immunity
- No/minimal pre-existing immunity
- Potential for distinguishing natural from vaccine immunity





Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4477–4481, May 1995
Microbiology

Recombinant vesicular stomatitis viruses from DNA

(rhabdovirus/viral replication/viral assembly)

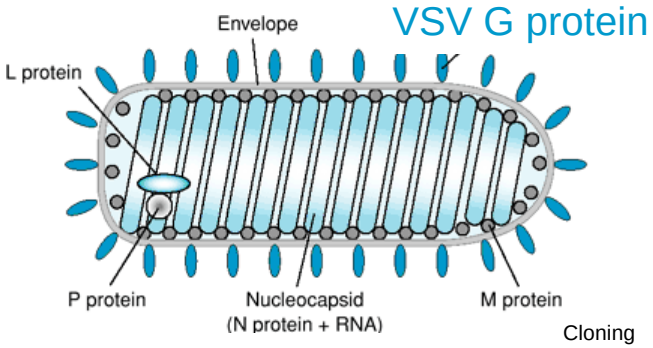
NATHAN D. LAWSON*†, ELIZABETH A. STILLMAN‡, MICHAEL A. WHITT§, AND JOHN K. ROSE*†§

Departments of *Pathology, §Cell Biology, and †Biology, Yale University School of Medicine, New Haven, CT 06510; and ‡Department of Microbiology and Immunology, University of Tennessee, Memphis, TN 38163

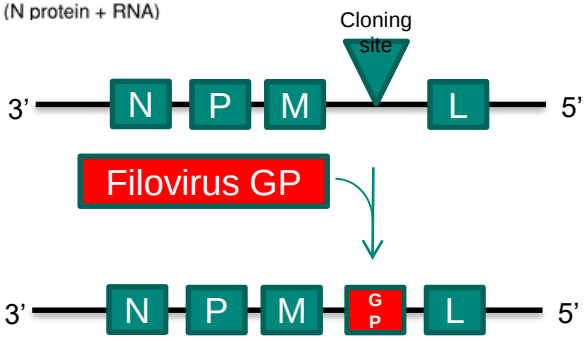
Advantages of VSV as a vector

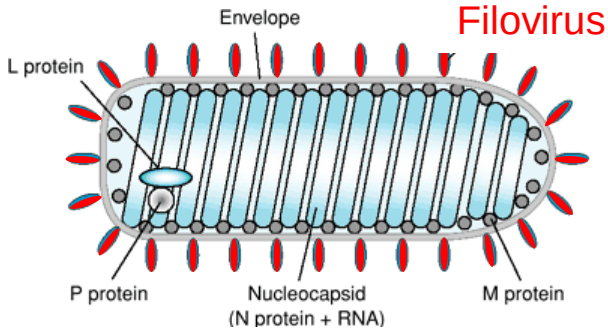
- Platform technology for multiple indications
- Attenuated by host restriction in humans
  - Wild type virus rare cause of disease in humans
- Replicating, rapid protection after a single dose
- Memory responses, durable immunity
- No/minimal pre-existing immunity
- Potential for distinguishing natural from vaccine immunity

Pseudotyping VSV with Ebola or Marburg virus GP



Complete replacement of VSV G protein (virulence factor, principal antigen) with desired target protein





Live, replication-competent recombinant vaccine

Garbutt M, et al. J Virol. 2004;78:5458-65

WHO R&D Blueprint

No presently existing medical countermeasures

- Crimean-Congo haemorrhagic fever
- Ebola virus and Marburg virus disease
- Lassa fever
- Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS)
- Nipah and henipaviral diseases
- Rift Valley fever
- Zika virus disease
- Disease X

Recombinant VSV vaccines against emerging and re-emerging diseases

Vaccine	Indication, status	Developer (funding)
rVSV-ZEBOV (V920)	Ebola, In registration	PHAC, NewLink, Merck (BARDA, DTRA), Profectus
rVSV-Marburg rVSV-Sudan	Marburg Sudan	Public Health Vaccines (BARDA)
rVSV-Lassa	Lassa fever	Emergent, Profectus, IAVI (CEPI)
rVSV-Nipah	Nipah	Public Health Vaccines
rVSV-CHIK	Chikungunya	Yale (NIAID)
rVSV-MERS	MERS	Harbin Veterinary Research Institute

VSV explored widely as a vector for other indications and as an oncolytic virus

Strong Preclinical Data Supports VSV as a Vector for Development of Ebola and Marburg Vaccines

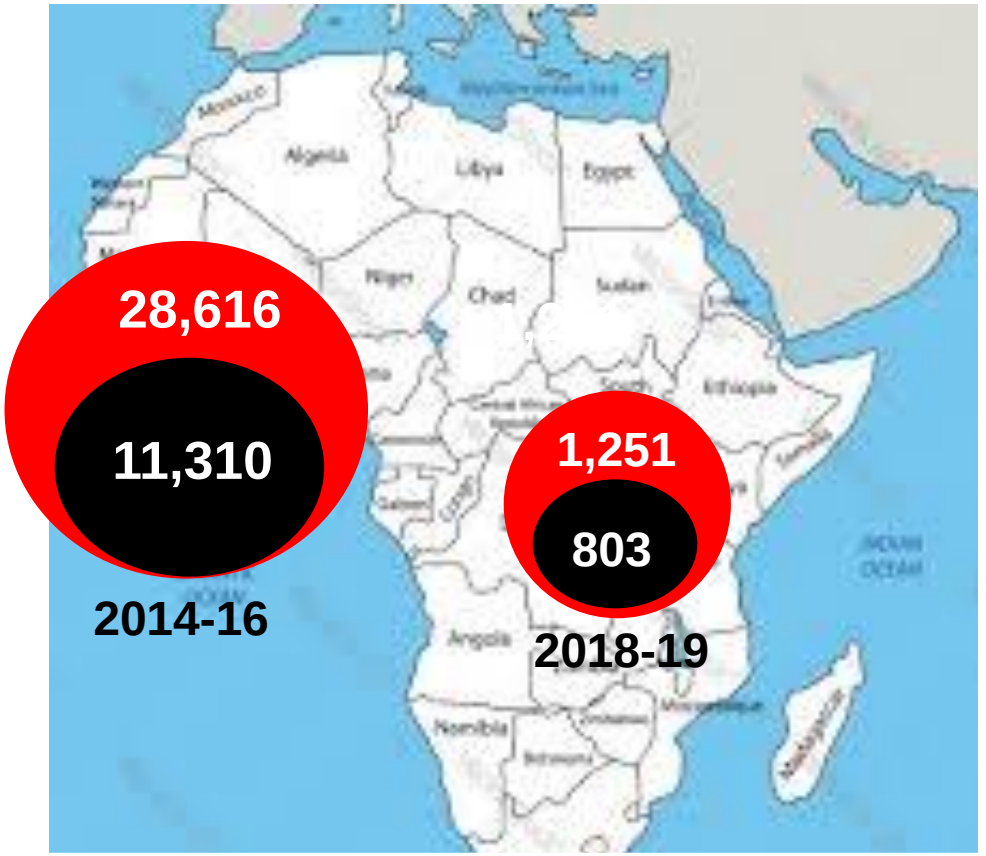
- 100% protection against lethal challenge (IM ~1000 LD_{50}) in NHPs following a single immunization
- Well tolerated in NHPs, minimal viremia
- Antibody response in 7-14 days
- Antibodies mediate protection
- Protection in NHPs with a single dose given shortly (3-7 days) before or even after challenge , mediated by innate immunity
- Safe, protective in immunodeficient (SHIV-infected) NHPs
- Not neurovirulent after IC inoculation in NHPs

rVSV as a Platform for Vaccine Development: Complete Protection in Non-human Primate Models

Vaccine	Dose(s) pfu	No. animals	Seroconvesion (ELISA, Neut)	Survival
rVSV-EBOV	$3x10^2$-$1x10^8$	45	100%	100%
Control		9	0%	0%
rVSV-MARV	$1x10^7$	18	100%	100%
Control		8	0%	0%
rVSV-Lassa	$1\text{-}3x10^7$	21	100%	100%
Control		10	0%	0%
rVSV-Nipah	$1x10^7$	3	100%	100% (0% ill)
Control		3	0%	67% (100% ill)

USAMRIID, Merck and NewLink, unpublished data
Feldmann H, unpublished data
Marzi A et al Emerg Infect Dis 215;21:305
Marzi A et al PNAS 2013:110:1893
Geisbert T et al PLoS Med 2005;2:e183
Geisbert T et al. Rev Med Virol 2010;20:344
Safronetz D et al. PLoS Negl Trop Dis 2015;9:e0003736
Prescott J et al Vaccine 2015;33:2823

rVSV-EBOV (V920, Merck) Vaccine



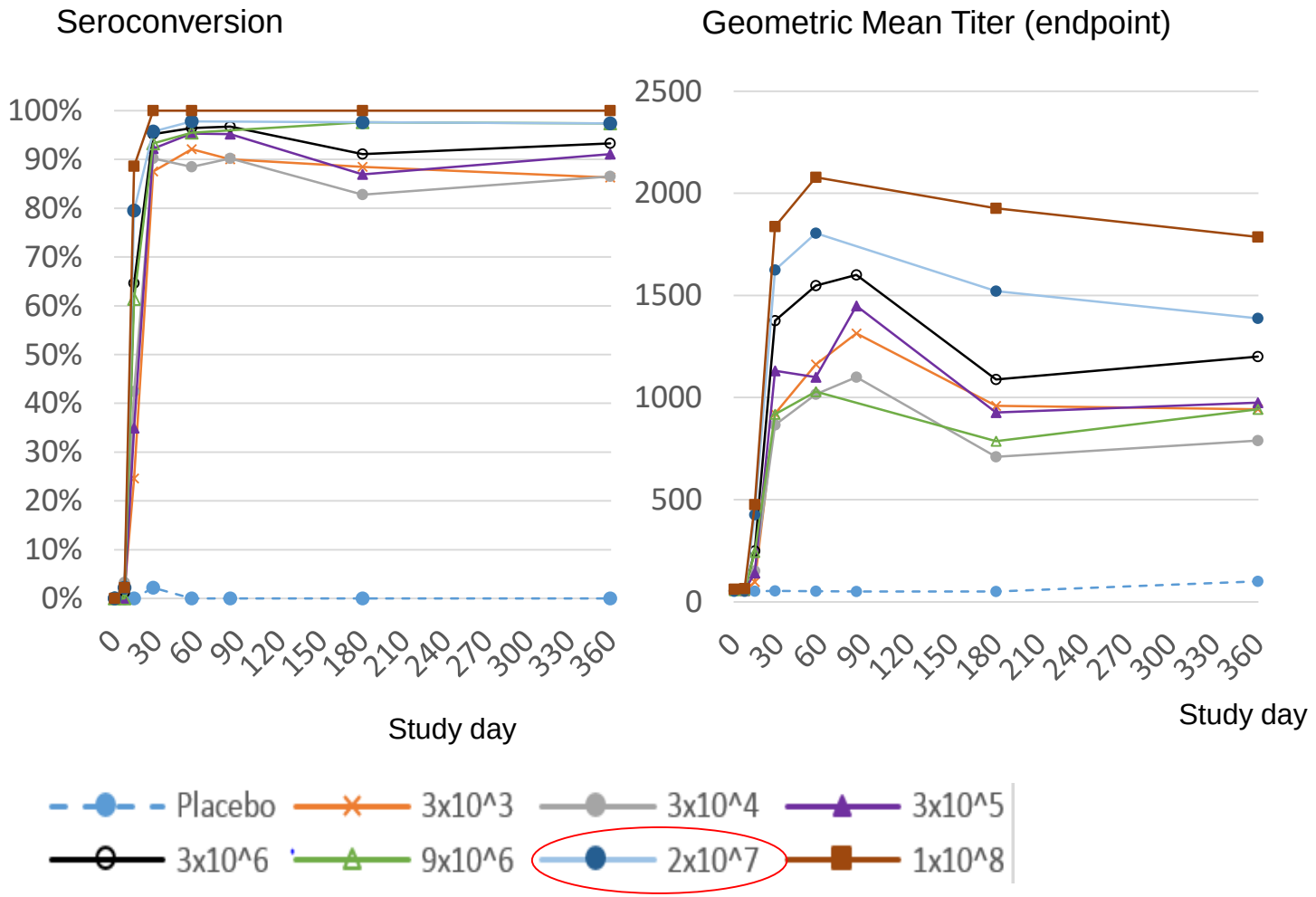
- >18000 people vaccinated in Phase I-III trials
- >75000 people vaccinated in expanded use protocol for outbreak control
- In registration US, EU

Antibodies mediate immunity by rVSV-EBOV (V920) vaccine

- Mixed effector functions play a role in protection (neutralization and Fc-mediated functional activities)
- Therapeutic efficacy with passive transfer of mAbs having both neutralizing and cell-targeting activities
- Active immunization fully protective despite CD8+ depletion
- Absence of non-survivors makes it difficult to differentiate immune correlates
- Antibody repertoire and epitope specificity in polyclonal response is poorly understood

rVSV-EBOV (V920, Merck) is highly immunogenic in humans across a wide dose range

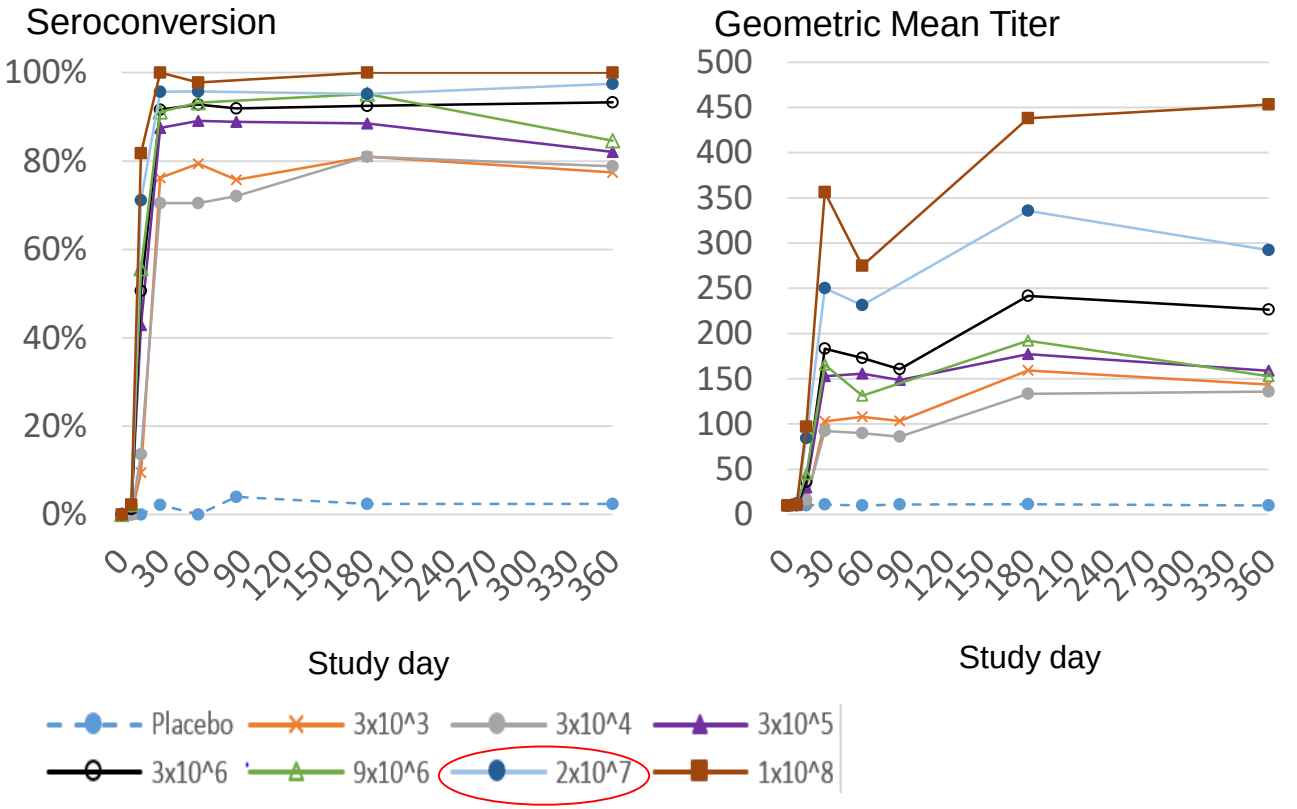
Phase 1b Clinical Trial (n=512) IgG ELISA



Heppner DG et al. *Lancet Infect Dis* 2017;17:854-866

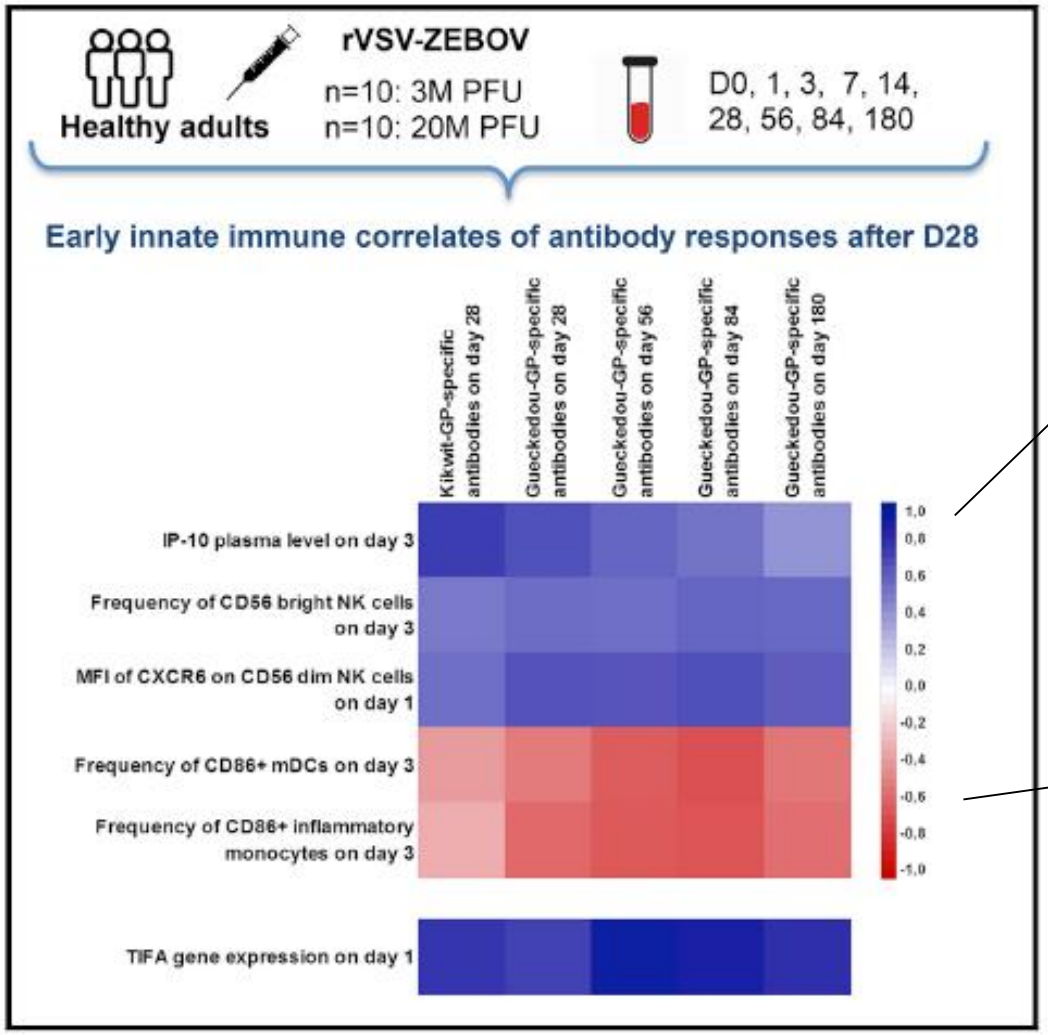
rVSV-EBOV (V920, Merck) is highly immunogenic across a wide dose range

Phase 1b Clinical Trial (n=512) 60% Plaque-reduction Neutralization



Heppner DG et al. *Lancet Infect Dis* 2017;17:854-866

Early innate response to rVSV-EBOV (V920) shapes and predicts adaptive humoral response



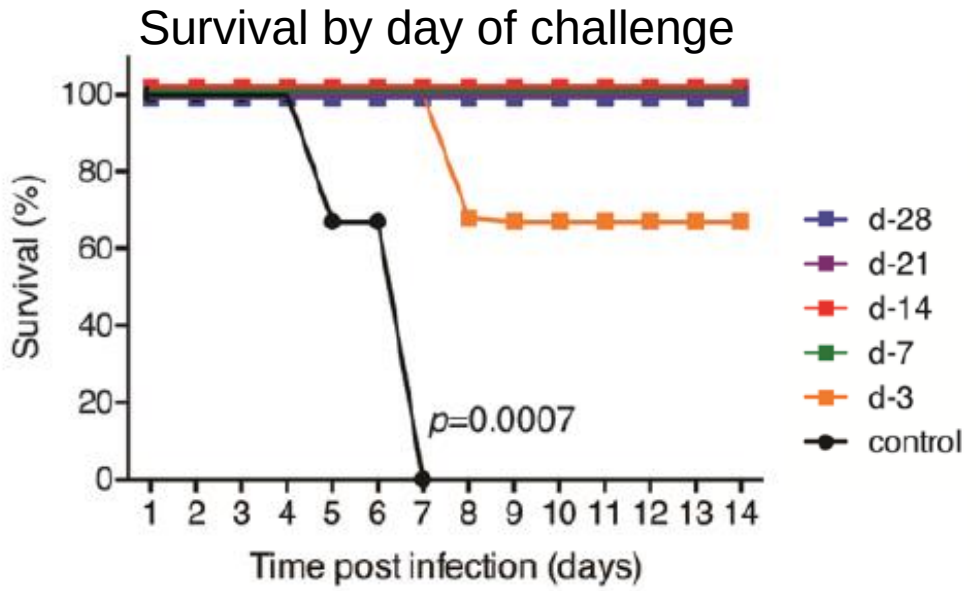
IP-10 and NK cells strong independent correlate of adaptive response

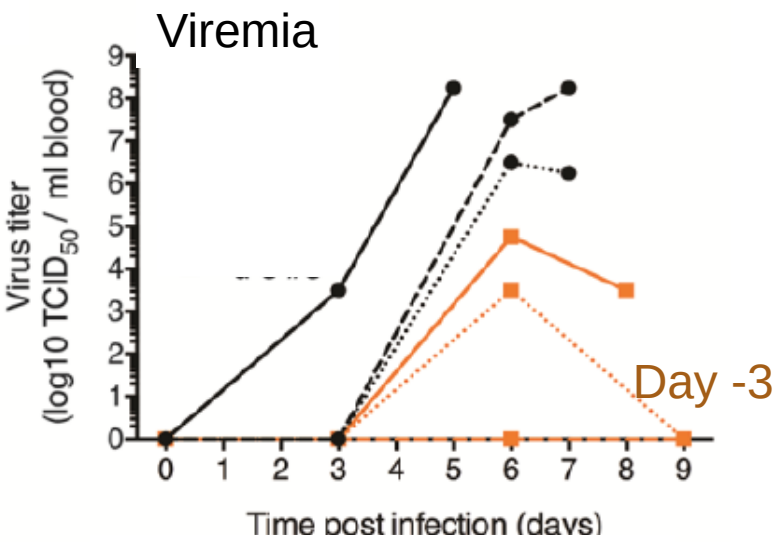
inflammatory Monocytes, mDCs expressing CD86 counter-regulatory, suppressive effect on adaptive response

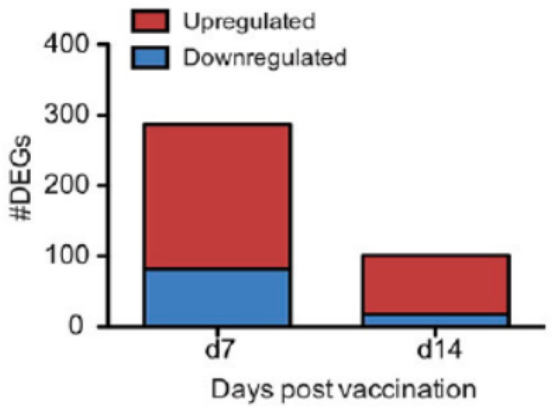
Rechtien et al., 2017, Cell Reports 20, 2251–2261

rVSV-EBOV Protects NHPs as early as 3 days before, or even briefly, after lethal challenge in absence of detectable adaptive immune response

Protection is not virus-specific and attributable to innate immunity





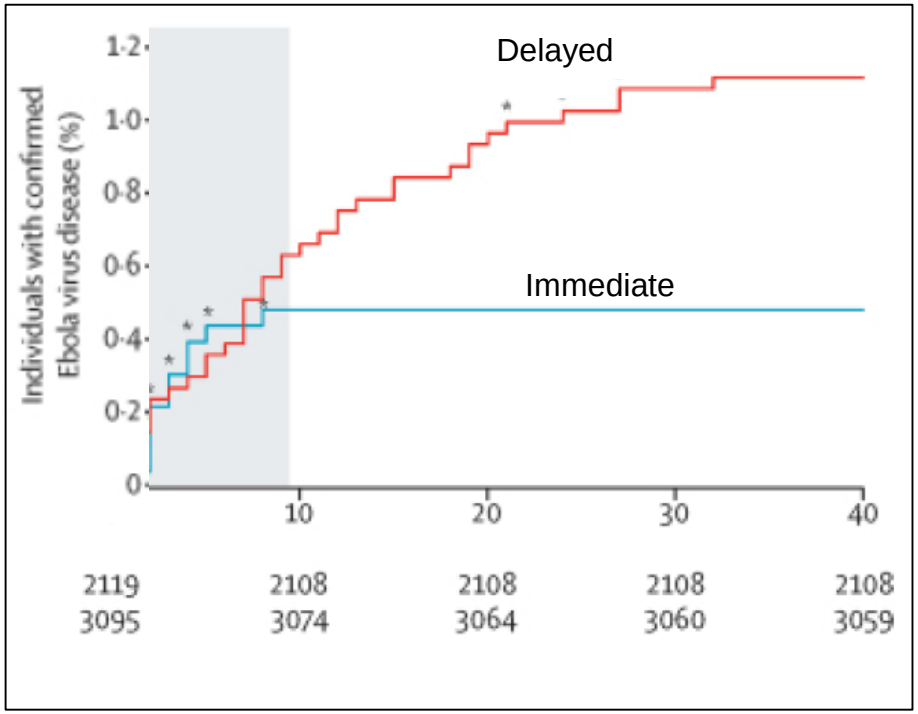


Marzi A et al. Science. 2015;349:739-42

Marzi A et al. Frontiers Immunol 2019;doi: 10.3399/fimmu.2018.03071

rVSV-EBOV (V920, Merck vaccine)
100% protective by 9 days after vaccination
Phase 3 “Ring Vaccination” trial, Guinea 2015

	Immediate Vaccina-tion	Delayed Vaccination
No. individuals (clusters)	2108 (51)	1429 (46)
Cases EVD	0	10
Attack rate	0%	0.7%
Vaccine efficacy	100% (p=0.045)	



Henao-Restrepo AM, et al. *Lancet. 2017;389:505-518.*.

FOR IMMEDIATE RELEASE
March 5, 2019

Contact: HHS Press Office
202-690-6343
media@hhs.gov

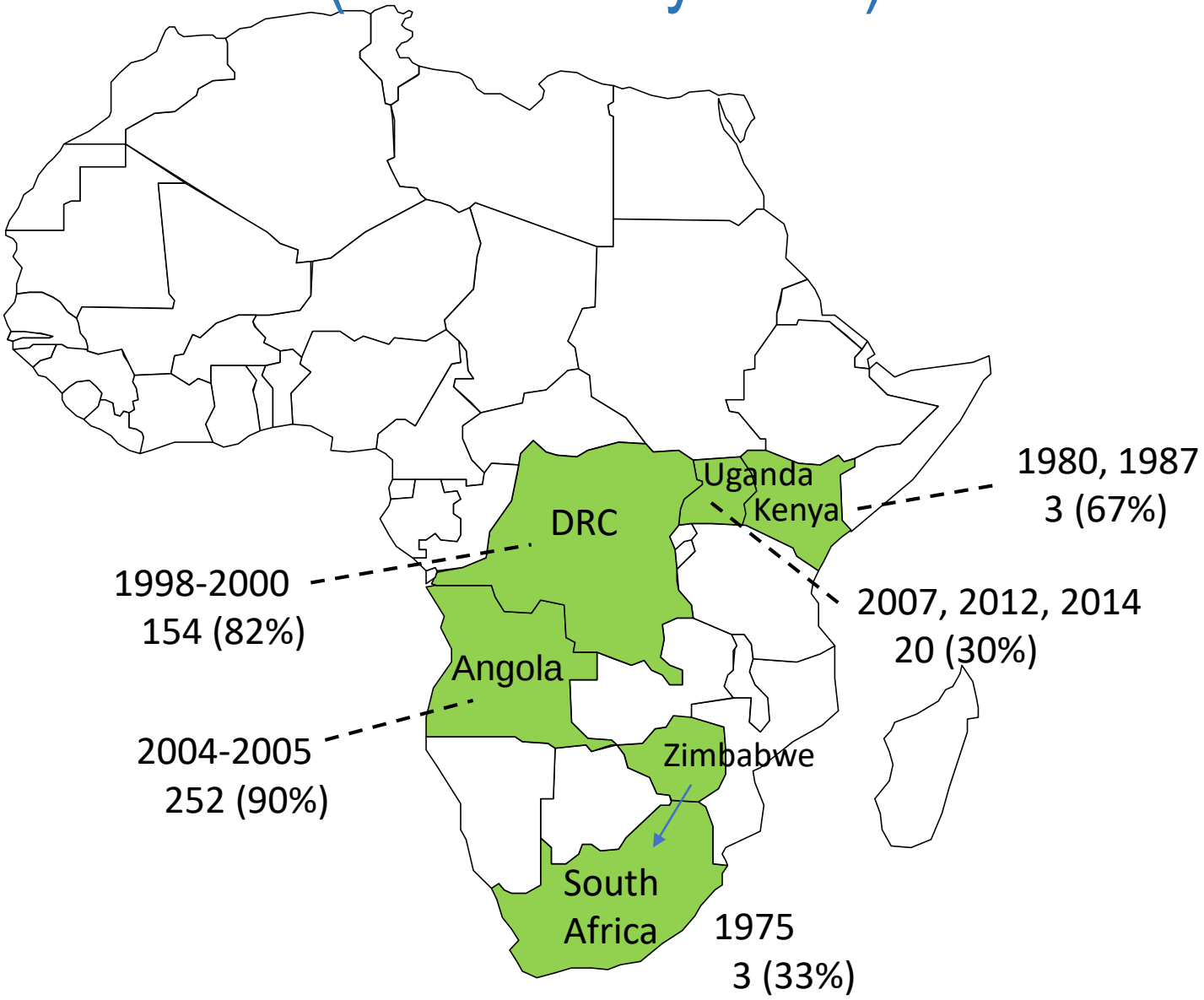
HHS' BARDA Funds Its First Marburg Virus Vaccine Development

Vaccine Could Address an Important Biodefense and Public Health Threat

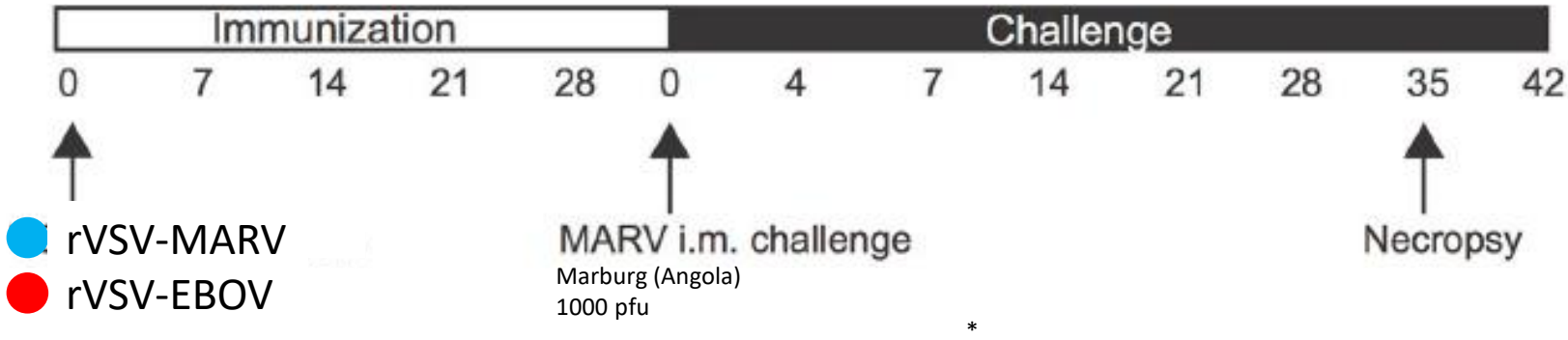
To increase national health security against biothreats and protect public health, the U.S. Department of Health and Human Services (HHS) will partner with Public Health Vaccines LLC of Cambridge, Massachusetts, to develop a potential vaccine against Marburg virus. No licensed vaccine for this virus exists today.

Marburg Virus disease

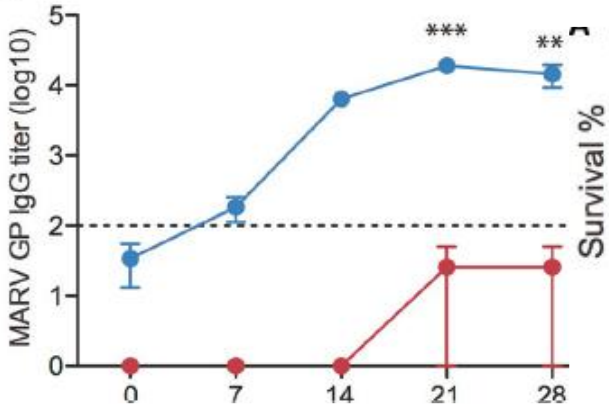
Location, no. cases (case fatality rates)



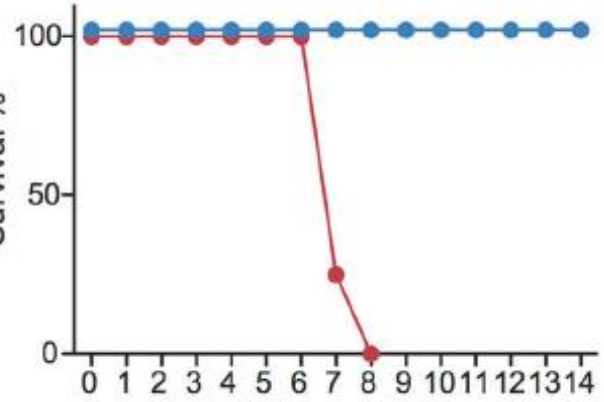
rVSV-Marburg: single dose protects cynos against Marburg Virus



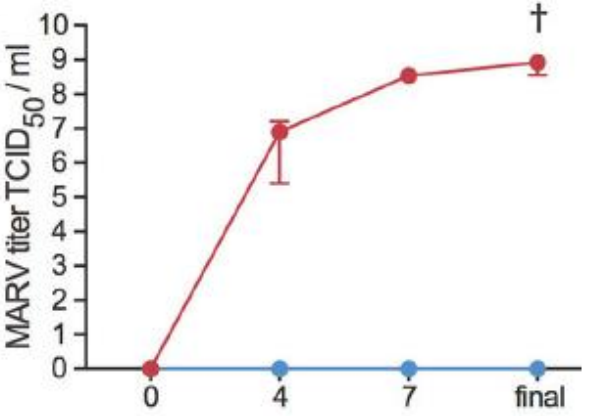
IgG ELISA



Survival



Viremia

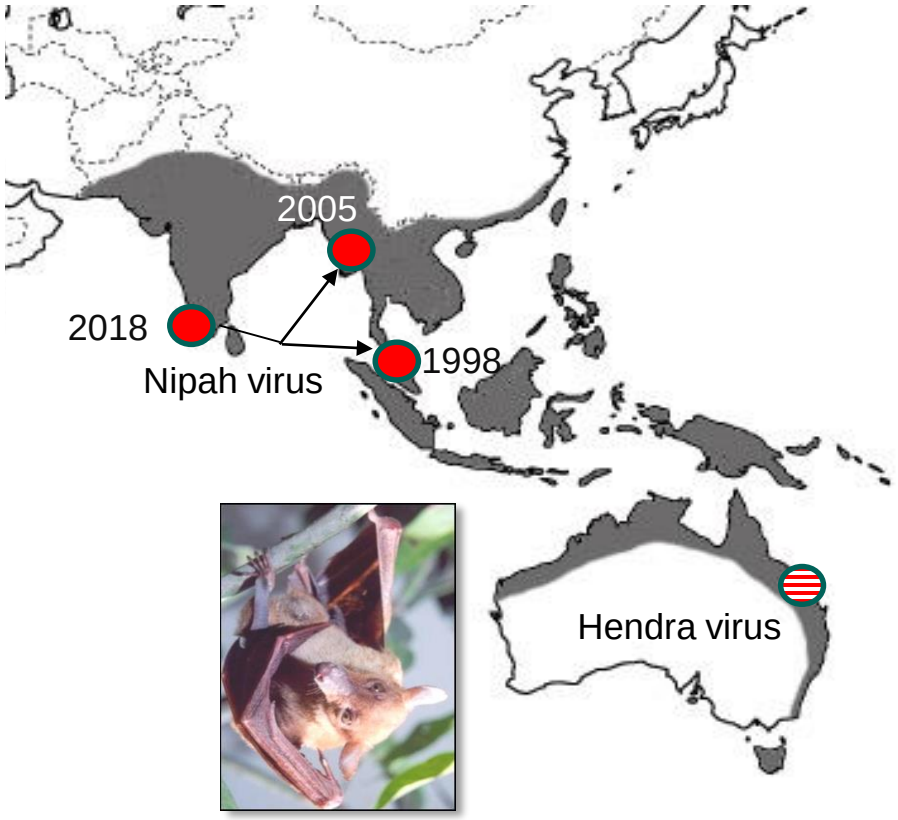


Marzi A et al. Frontiers Immunol 2019;doi: 10.3399/fimmu.2018.03071

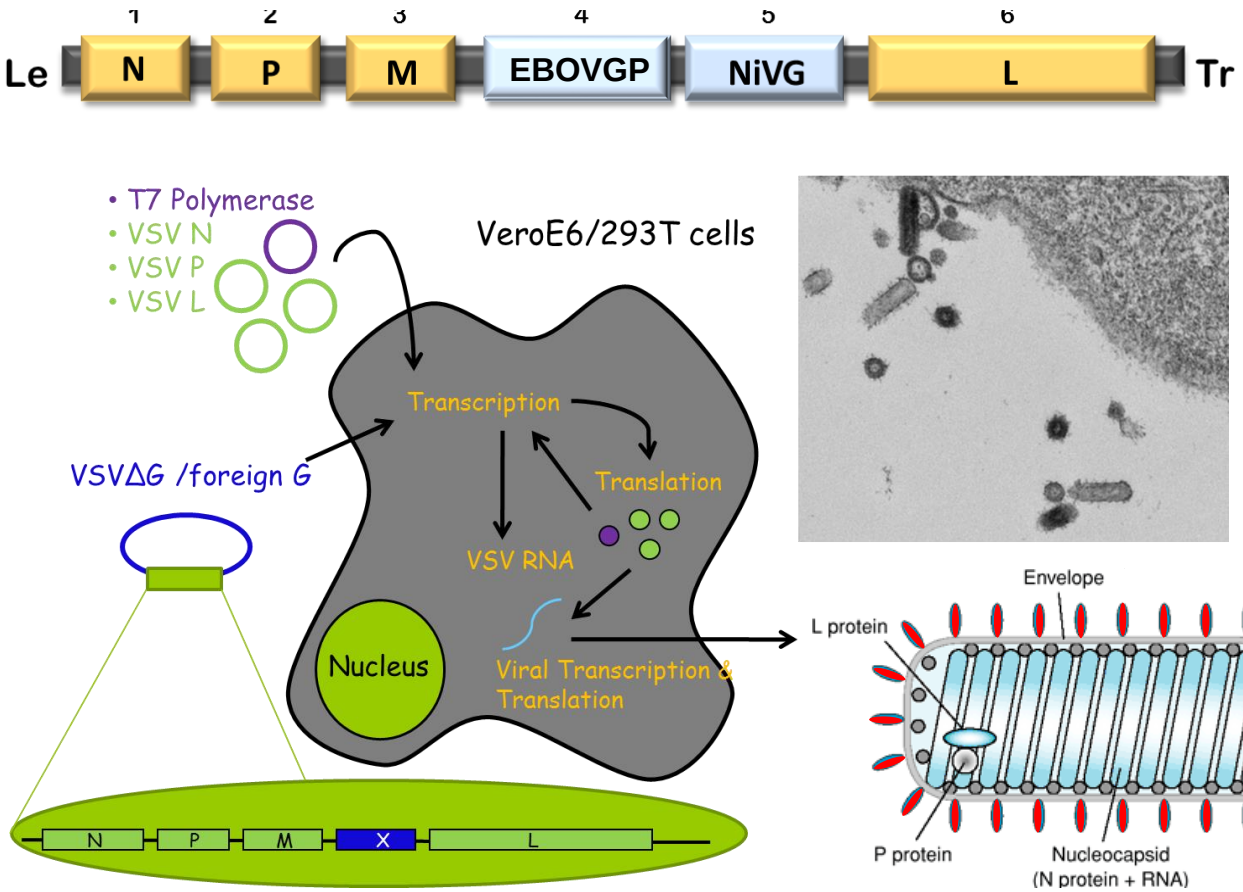
Nipah Virus Disease

- Paramyxovirus
- Sporadic/epidemic
- 10-50 cases/year
- SE Asia, India
- *Pteropus* bat reservoir
- Pigs affected and may be source of human infection
- Encephalitis, vasculitis, pneumonitis
- 70% CFR

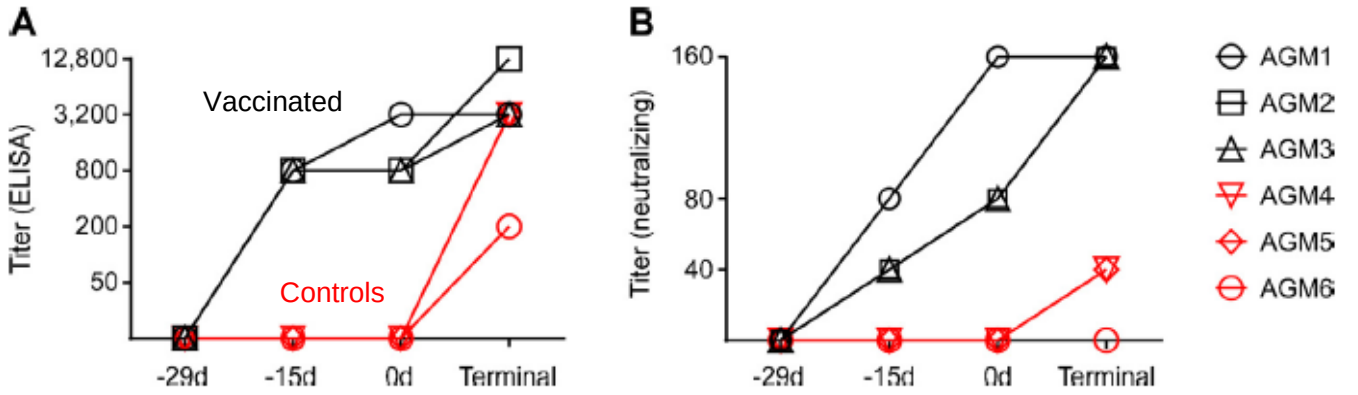
- Closely related Hendra virus in Australia
- Commercial subunit Hendra vaccine for horses
- Vaccination of horses protects humans from contact infection

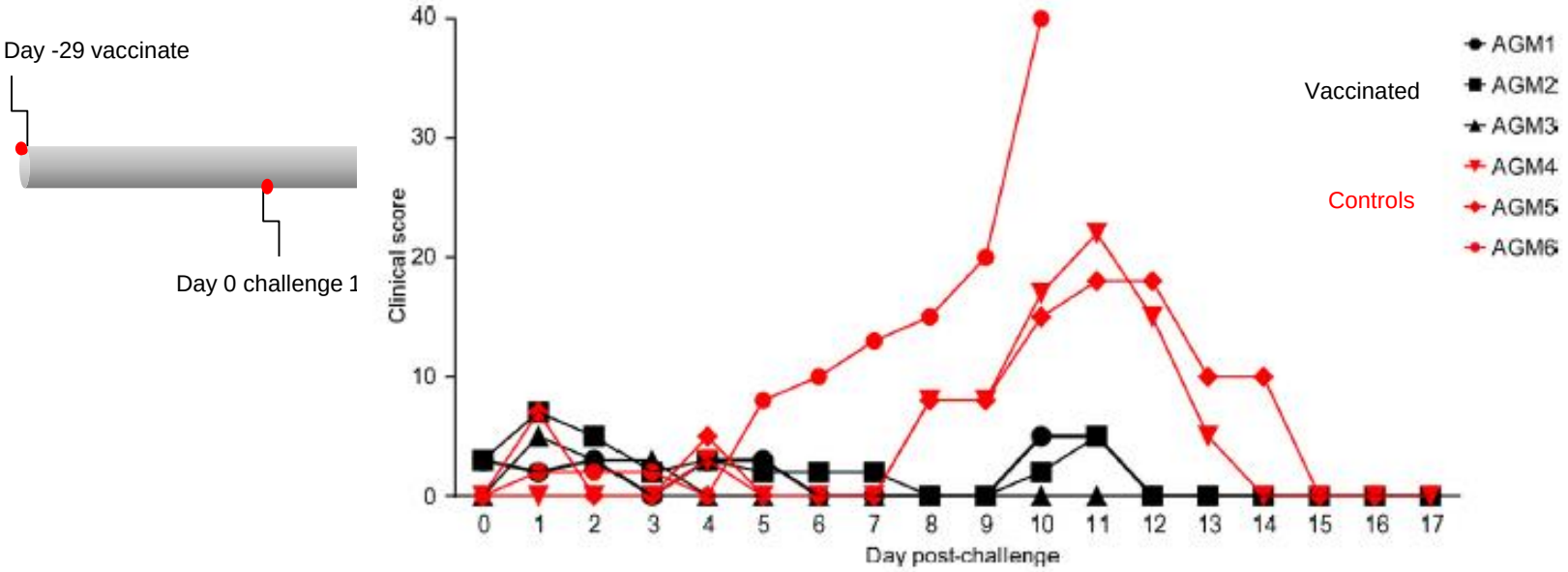


Reverse genetics system for generating rVSV Nipah candidate vaccine



Single dose of rVSVΔG/ZEBOVGP/NiVG elicits strong humoral response and protects African Green Monkeys





Prescott J et al. Vaccine 2015;33:2823

ANIMAL VIRUS VECTOR PLATFORMS FOR VETERINARY APPLICATIONS

Live, replicating recombinant vaccines for veterinary use

- Requirements
  - Host range covers target species
  - Carrying capacity for nonessential foreign gene(s) of interest
  - Immunogenic
  - Safe

- Alphaviruses
- Pox viruses
- Herpes viruses, e.g. pseudorabies (PRV)
- BVDV
- Rhabdoviruses
  - SAD B19-based RABV
  - ???VSV

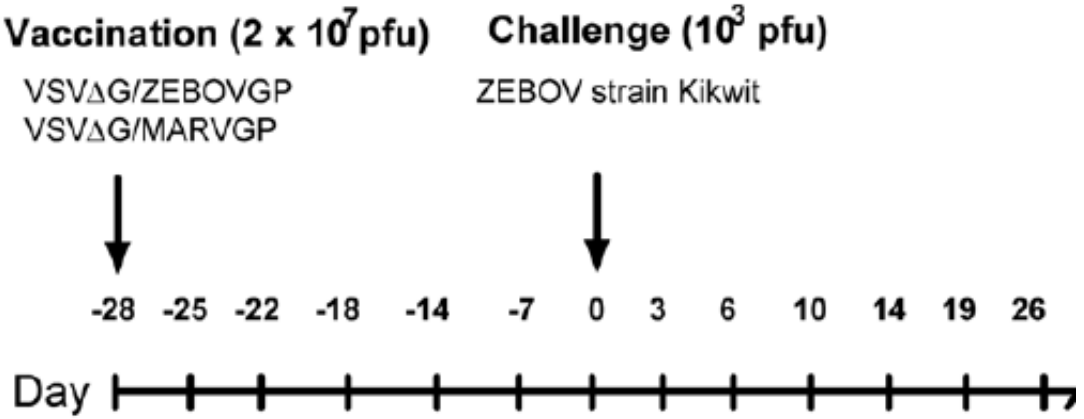
Commercial recombinant vectored veterinary vaccines using animal virus vectors

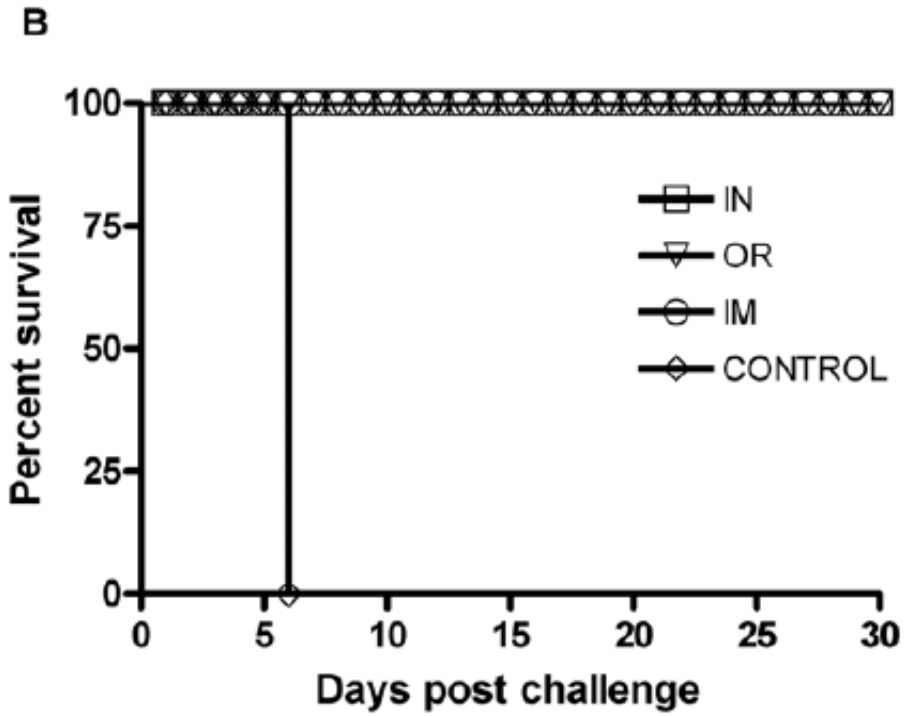
Species	Pathogen	Type
Cat	Feline leukemia	Canary pox
Cat	Rabies	Canary pox
Dog	Distemper	Canary pox
Ferret	Distemper	Canary pox
Horse	Influenza	Canarypox (MVA, herpes-1)
Horse	West Nile	Yellow fever chimera, vaccinia
Poultry	Avian influenza, Newcastle's, Infectious laryngotracheitis	Fowlpox, Turkey herpesvirus
	Infectious bursal disease	Fowlpox
Swine	Classical swine fever	Recombinant bovine viral diarrhea chimera
	Circovirus	Canary pox

VSV as a Vector for Animal Vaccines?

- Wide Host range
  - Virtually all mammalian species
- Accommodates large and multiple foreign gene inserts, without affecting replication
- Proven as human viral vector
- Potential for DIVA vaccine
- Potential for mucosal delivery

rVSV-EBOV administered orally, intranasally or by IM injection protects cynos against lethal EBOV challenge





Qiu X et al PLoS One 2009; 4(5):e5547

rVSV-EBOV Vaccine for Immunization of Great Apes against Ebola

- Catastrophic loss of >50,000 gorillas and many chimpanzees due to Ebola epizootics 1990s and early 2000s
- Current Ebola outbreak in northeastern DRC threatens gorilla populations including critically endangered Mountain gorillas (*Gorilla beringei*)
- Preparations made to vaccinate habituated gorillas with rVSV-EBOV (V920, Merck) by dart gun

Chimp killed by Ebola, Rep Congo 2014
Walsh P et al. Sci Rep, 2017

SCIENTIFIC REPORTS

OPEN

SAD B19 RAB-EBOV

The Final (Oral Ebola) Vaccine Trial on Captive Chimpanzees?

Peter D. Walsh[1], Drishya Kurup[2], Dana L. Hasselschwert[3], Christoph Wirblich[2], Jason E. Goetzmann[3] & Matthias J. Schnell[2]

Received: 19 July 2016
Accepted: 26 January 2017
Published: 09 March 2017

Could new oral vaccine technologies protect endangered wildlife against a rising tide of infectious disease? We used captive chimpanzees to test oral delivery of a rabies virus (RABV) vectored vaccine against Ebola virus (EBOV), a major threat to wild chimpanzees and gorillas. EBOV GP and RABV GP-specific antibody titers increased exponentially during the trial, with rates of increase for six orally vaccinated chimpanzees very similar to four intramuscularly vaccinated controls. Chimpanzee sera also showed robust neutralizing activity against RABV and pseudo-typed EBOV. Vaccination did not induce

OPEN

RhCMV-EBOV

Cytomegalovirus-based vaccine expressing Ebola virus glycoprotein protects nonhuman primates from Ebola virus infection

Andrea Marzi[1,*], Aisling A. Murphy[2,*], Friederike Feldmann[3], Christopher J. Parkins[4], Elaine Haddock[1], Patrick W. Hanley[3], Matthew J. Emery[5], Flora Engelmann[6], Ilhem Messaoudi[6], Heinz Feldmann[1,#] & Michael A. Jarvis[2,#]

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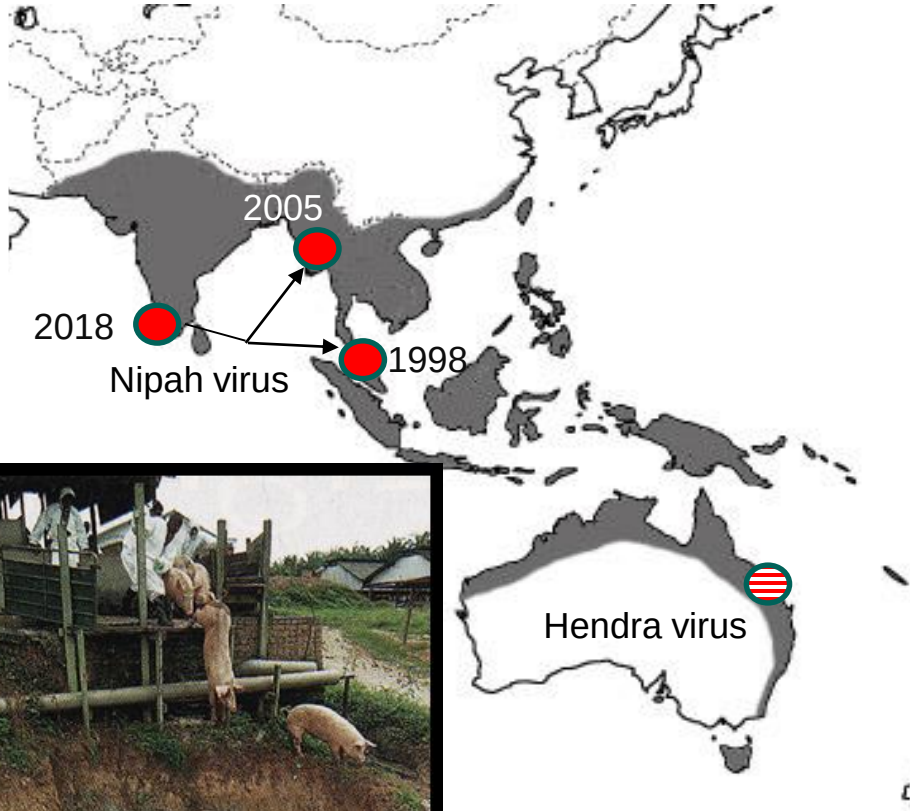
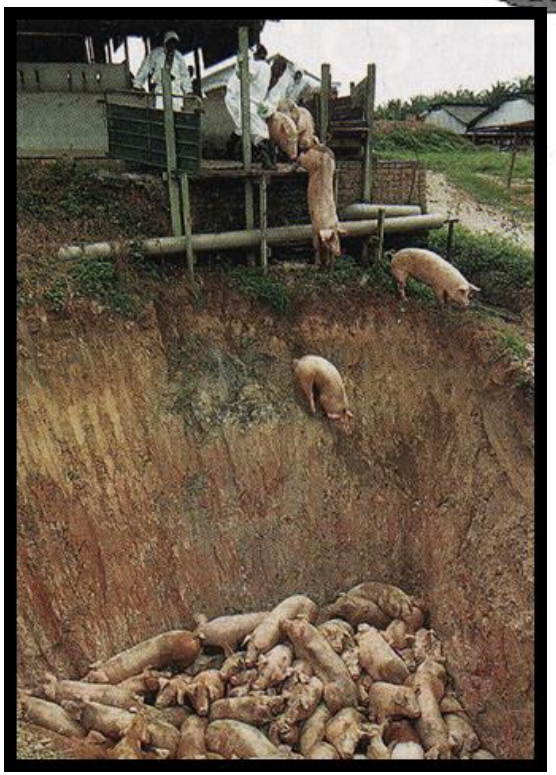
Ebolaviruses pose significant public health problems due to their high lethality, unpredictable emergence, and localization to the poorest areas of the world. In addition to implementation of standard public health control procedures, a number of experimental human vaccines are being explored as a further means for outbreak control. Recombinant cytomegalovirus (CMV)-based vectors

Nipah Virus Disease

- The 1998 outbreak in Malaysia was concentrated among pig farmers
  - 92% of cases reported contact with pigs
- Compared to controls, persons with Nipah encephalitis were
  - 5.6 X more likely to have close contact with pigs.
- Outbreak ceased following culling over 900,000 pigs
  - Pork industry decimated
- Vaccination of swine a feasible control measure?





Courtesy: S Luby

BBC news

Safety of recombinant VSV constructs for animal species susceptible to VSV disease

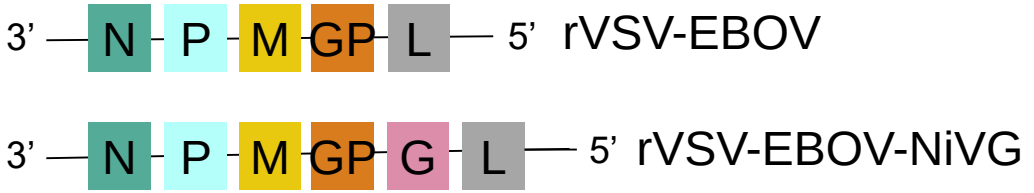
- Host range and virulence factors
  - Virus replication dependent on vector (VSV) genes
  - VSV has very wide host range in all mammals
  - Cell/tissue tropism dependent on transgene

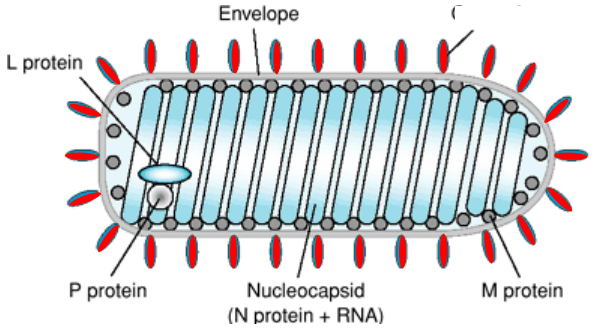
Ebola broad species tropism
spans 6 mammalian orders

- Bats
- Humans
- Monkeys
- Pigs
- Duiker
- Cats
- Dogs
- Ferrets
- Mice
- Hamsters
- Guinea pigs

Ebola enters mammalian cells through the Niemann-Pick C1 receptor

High sequence homology of NPC-1



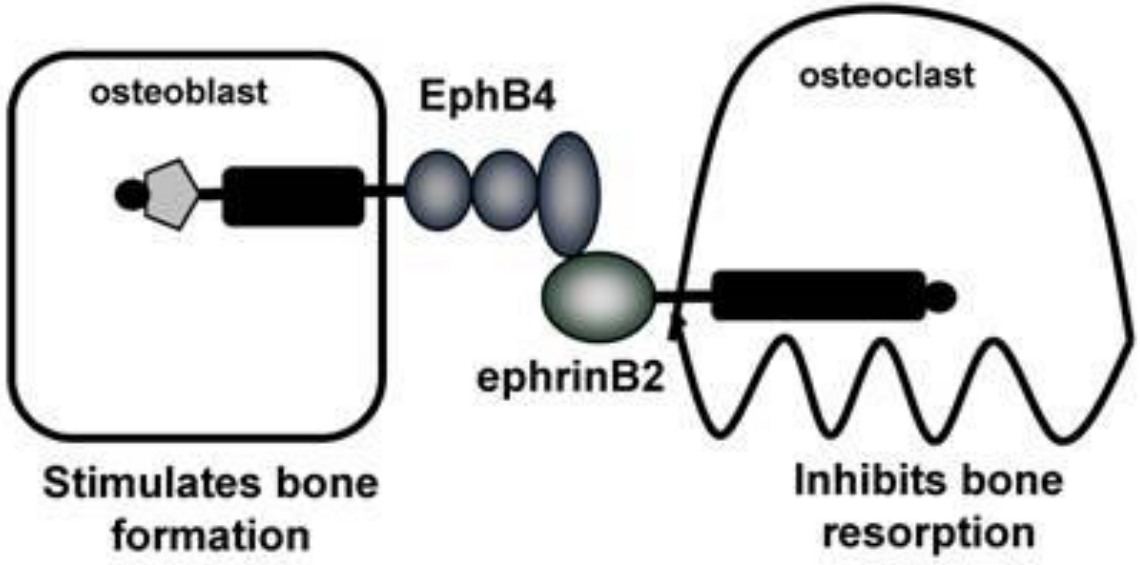


Henipavirus (Nipah) broad species tropism spans 6 mammalian orders

- Bats
- Humans
- Monkeys
- Pigs
- Horses
- Cats
- Dogs
- Ferrets
- Hamsters
- Guinea pigs

Nipah enters mammalian cells through the ephrin-B2 or ephrin-B3 surface glycoprotein

95-98% sequence homology of ephrin-B2/B3 across mammals



Int J Med Sci 2008; 5(5):263-272

Safety of recombinant VSV constructs for animal species susceptible to VSV disease

- In a study of pigs inoculated (oral, snout) with wild-type VSV or rVSV-ZEBOV, the majority of animals developed VSV-like clinical illness
  - Illness delayed and milder in rVSV-ZEBOV group
- Conclusions
  - rVSV safety for livestock needs careful evaluation in target species, with consideration of the transgene host range

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Monath TP et al1. Vaccine X 2019:100009

Other Vesiculovirus Vectors

- Closely related viruses with similar genome organization
- Vesiculovirus genus with no known illness in humans or domestic livestock that could serve as vectors
  - Isfahan --Jurona --Malpais Spring
  - Carajas -- Maraba --Perinet

Recombinant Isfahan Virus and Vesicular Stomatitis Virus Vaccine Vectors Provide Durable, Multivalent, Single-Dose Protection against Lethal Alphavirus Challenge

J Virol 2017:91:e01729-16

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Conclusions

- One Health Paradigm illustrates opportunities for:
  - Vaccination of animals to prevent certain zoonotic infections of humans
  - Use of animal viruses for design and of vaccines and their application
- Replicating vaccines have advantages for immunogenicity, rapid intervention, durability of protection
- Host range and cell tropism critical to in vaccine design
- Multiple animal virus vectors being clinically tested in humans and some are commercialized or in registration (Pox, Flavi, Rota, VSV)
- VSV a prominent candidate for human vaccines, but safety concerns remain for veterinary applications
- Nonpathogenic VSV-relatives are in development as vectors

Thank you!