Evaluation of the Transmission Potential of Two WEE Vaccine Candidates

Joanie Kenney

October 20, 2009

ABSA, Miami, Fl

Talk Outline

- Alphavirus/WEEV Background
- Experimental Background
  - WEEV vaccines in mice (Atesheva et al.)
  - Objectives
  - Hypothesis
- Methods
  - Mosquito rearing
  - Experimental
- Results
- Conclusions and future directions

Medically Important Alphaviruses

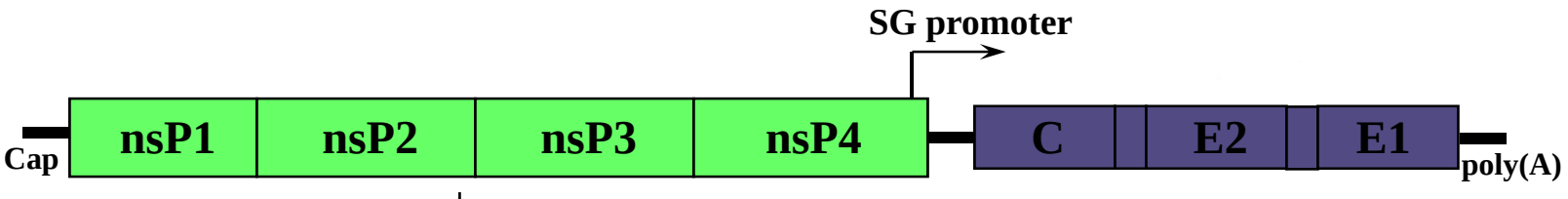
Encephalitic

- Venezuelan equine encephalitis
- Eastern equine encephalitis
- Western equine encephalitis

Arthralgic

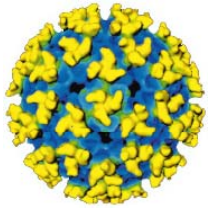
- Sindbis
- Chikungunya
- Semliki Forest
- Ross River
- Mayaro
- Barmah Forrest
- O’nyong’nyong

Alphavirus Structure



Development of innate immune response
Determine virus-host interactions

Development of adaptive immune response (structural proteins)



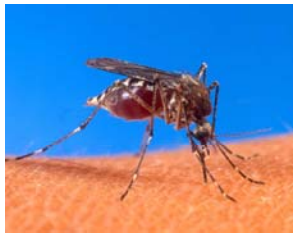
- Positive sense RNA virus of about 11.5 KB

WEE Transmission Cycle

House Finch

Cx. tarsalis

Cx. tarsalis



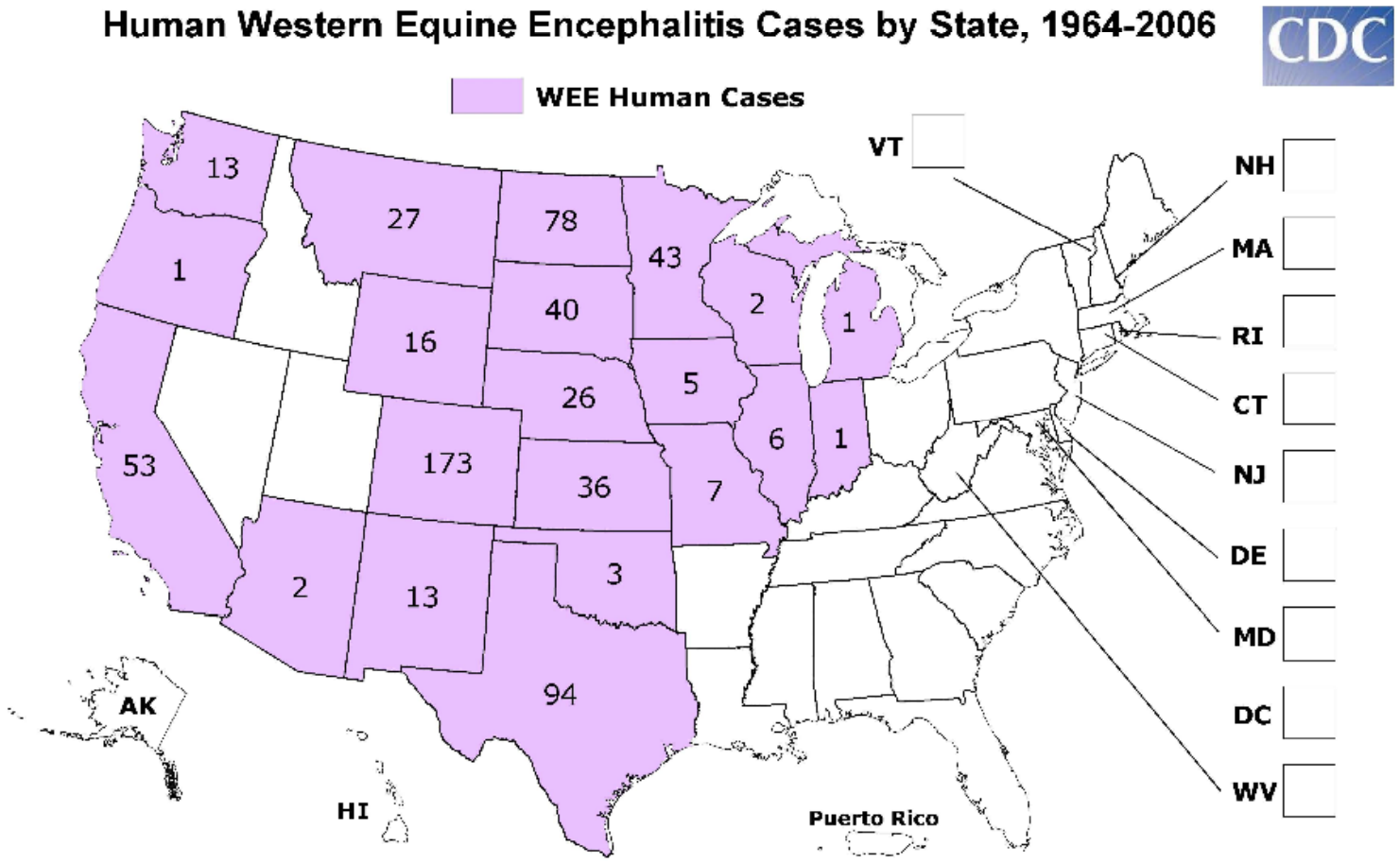
Ae. melanimon

House sparrow

Rodents

Dead-end hosts

WEE Distribution



WEE Epidemiology

- WEE activity
  - Major epizootics from 1930 - 1938 affected over 300,000 horses (Davison 1941; Meyer et al. 1930)
    - Saskatchewan, Canada alone had 52,000 horse cases with 15,000 horse deaths
  - In 1941 over 1,094 human cases in Canada and 2,242 in US
    - Involved states included both Dakotas, Minnesota, Nebraska
  - In Argentina, WEE was isolated in 1933 (Rosenbush 1934)
    - Human WEE occurred in 1972 and 1983 (rare) (Calisher et al. 1985).

Disease in Humans

- WEEV in humans can manifest as a full-blown encephalitic disease, a milder general flu-like illness, or as a subclinical infection
  - Ratio of unapparent to apparent infection starts off low (i.e. 1:1) for children under 1yr and grows to 1150:1 in children over 4 yrs.

Disease in Domestic Animals

- Typically referring to horses
  - WEEV disease is similar to illness caused by EEE or VEE
- Consists of a 1-3 week incubation
- Signs include fever, anorexia, restlessness, irritability, decreased locomotion and ataxia
  - Severe disease consists of circling and eventual signs of brain dysfunction (stupor, drooping of the head, inability to stand, blindness, flaccidity of the lips, excessive salivation, partially closed eyelids, convulsions, and paralysis) (Monath and Trent 1981)

Prophylaxis

- Vector Control
- Vaccines
  - In development
    - DNA candidate – requires 2 boosters
    - Adenovirus vectored vaccine candidates
  - In use (veterinary)
    - Killed trivalent veterinary vaccines (VEEV/WEEV/EEEV)

Our Goal

- Develop a genetically stable live-attenuated vaccine that is safe, immunogenic, and protective
  - Pros
    - Long lasting immunity (one dose)
  - Cons
    - Potential for reversion

Our Strategy

Chimeric alphavirus vaccine candidates protect mice from intranasal challenge with western equine encephalitis virus

Svetlana Atasheva[a,1], Eryu Wang[b,c,d,1], A. Paige Adams[b,c,d,1], Kenneth S. Plante[b,c,d], Sai Ni[b,c,d], Katherine Taylor[b,c,d], Mary E. Miller[a], Ilya Frolov[a,b], Scott C. Weaver[a,b,c,d,*]

[a] Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555, United States
[b] Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, TX 77555, United States
[c] Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555, United States
[d] Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX 77555, United States

ARTICLE INFO

Article history:
Received 10 February 2009
Received in revised form 27 April 2009
Accepted 4 May 2009
Available online 27 May 2009

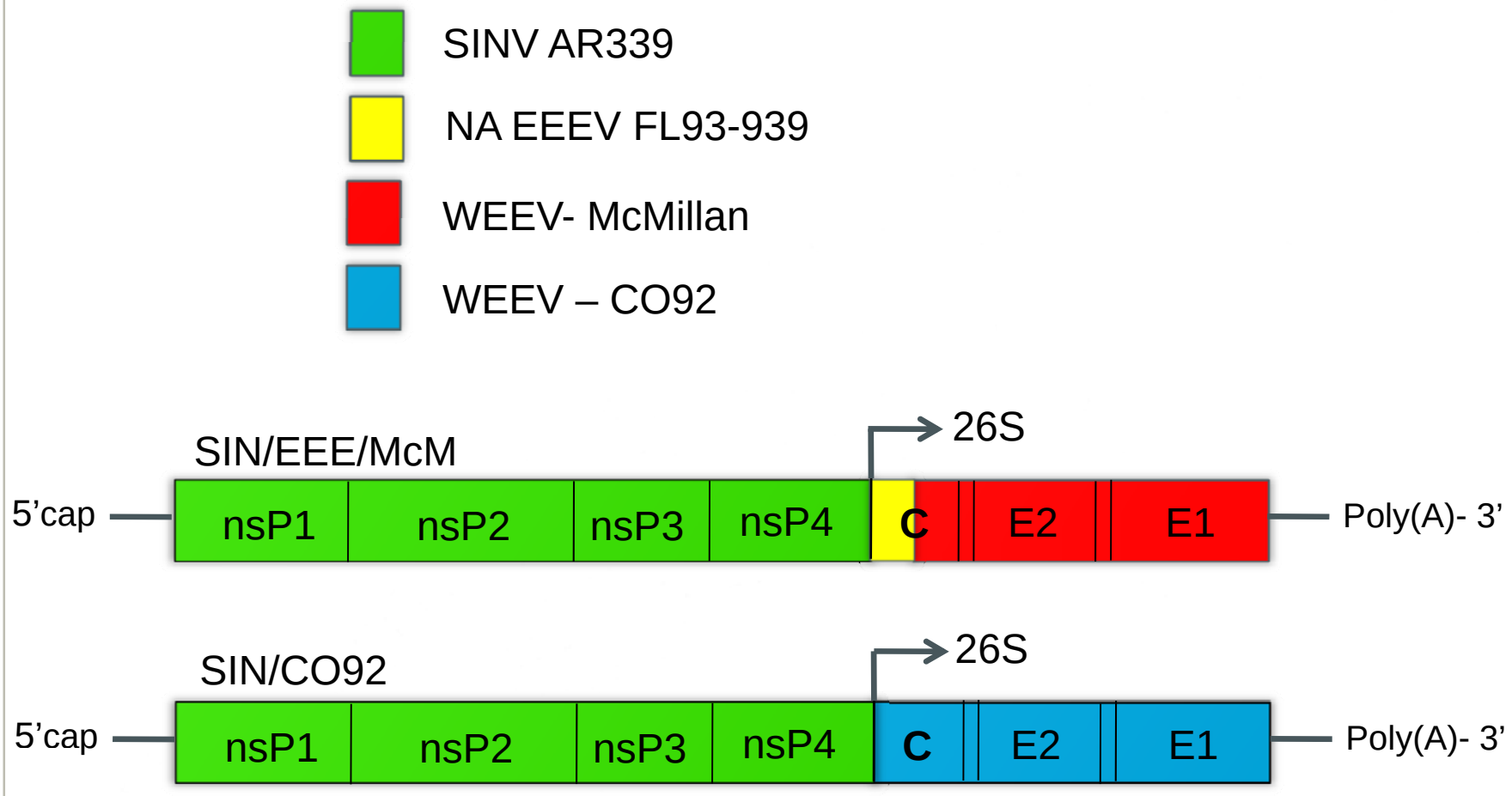
Keywords:
Alphavirus
Western equine encephalitis virus
Vaccine
Virulence

ABSTRACT

We developed two types of chimeric Sindbis virus (SINV)/western equine encephalitis virus (WEEV) alphaviruses to investigate their potential use as live virus vaccines against WEE. The first-generation vaccine candidate, SIN/CO92, was derived from structural protein genes of WEEV strain CO92-1356, and two second-generation candidates were derived from WEEV strain McMillan. For both first- and second-generation vaccine candidates, the nonstructural protein genes were derived from SINV strain AR339. Second-generation vaccine candidates SIN/SIN/McM and SIN/EEE/McM included the envelope glycoprotein genes from WEEV strain McMillan; however, the amino-terminal half of the capsid, which encodes the RNA-binding domain, was derived from either SINV or eastern equine encephalitis virus (EEEV) strain FL93-939. All chimeric viruses replicated efficiently in mammalian and mosquito cell cultures and were highly attenuated in 6-week-old mice. Vaccinated mice developed little or no detectable disease and showed little or no evidence of challenge virus replication; however, all developed high titers of neutralizing antibodies. Upon intranasal challenge with high doses of virulent WEEV strains, mice vaccinated with $\geq 10^5$ PFU of SIN/CO92 or $\geq 10^4$ PFU of SIN/SIN/McM or SIN/EEE/McM were completely protected from disease. These findings support the potential use of these live-attenuated vaccine candidates as safe and effective vaccines against WEE.

Live-Attenuated Chimeras

(More genetically Stable)



Chimeric Vaccines in Mice

- Attenuation
  - Both SIN/CO92 and SIN/EEE/McM resulted in no viremia nor overt disease after sc inoculation of up to 5.0 $\log_{10}$ PFU
- Immunity
  - 4 weeks post sc dose, all animals in both groups showed neutralization at $PRNT_{80}$
- Protection
  - Challenged 28 dpi with either TBT 235 or McMillan, all survived with no apparent disease

Ecological Safety

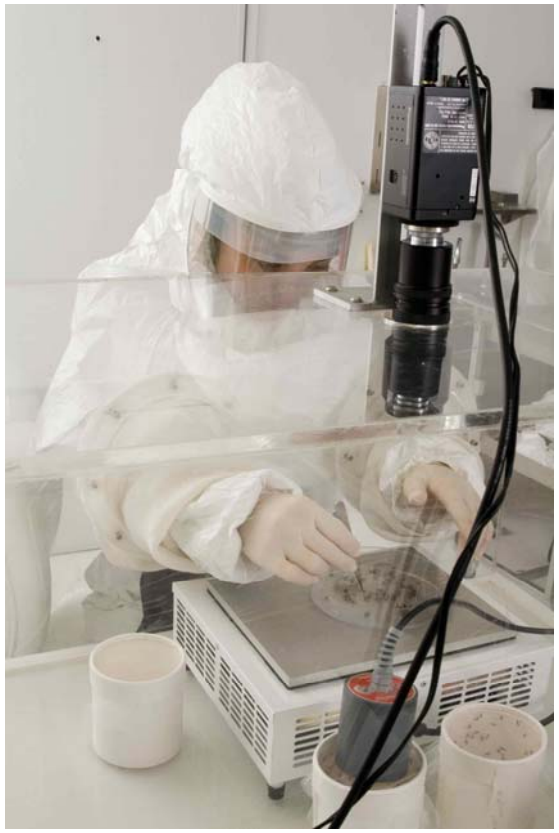
- If a human or equid vacinee happened to become viremic, could a mosquito acquire and transmit the vaccine strains?
  - If so, would the vaccine strain remain attenuated?
- **Can these vaccine strains infect, disseminate, and be transmitted by *Cx. tarsalis?***

Hypothesis

- HO: That the attenuated chimeric vaccine candidates will show a diminished rate of infection and dissemination in the mosquito vector, *Cx. tarsalis* as compared to wild type WEEV
- HO: *Cx. tarsalis* will be unable to transmit either vaccine candidate to a naïve host

Working With WEEV and Mosquitoes

- WEEV, VEEV, EEEV are highly aerosolizable with over 160 lab infections
  - BSL3 (enhanced)
- Not a select agent (VEEV, EEEV are select agents)
- No experimental vaccine
- Additional hazards of working with these viruses require additional respiratory PPE and HEPA filtered exhaust
  - PAPR, half face cartridge respirators



Propagating Mosquitoes

- First we must acquire the species
  - From other colonies (i.e. Dr. Reisen, UC Davis)
  - Collect and colonize
- To propagate, we nurse them through the life stages
- Prior to an experiment we sort out females and starve for 24 hours
  - Adult females feed on blood, adult males typically feed on nectar

Propagating Mosquitoes

Eggs

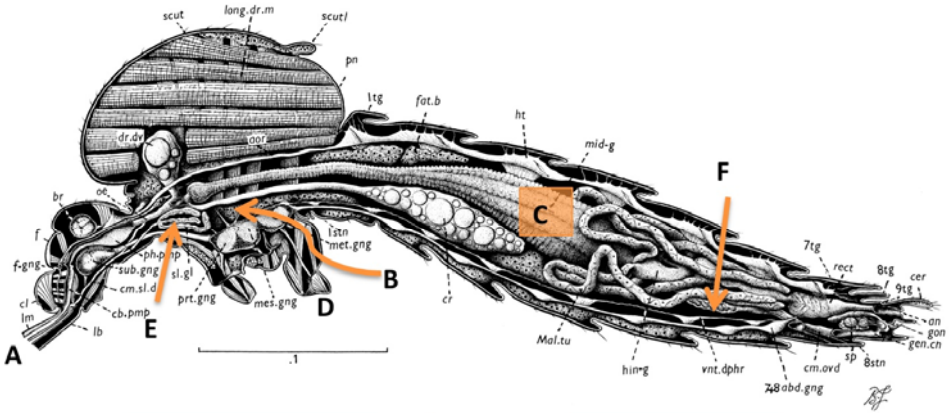
Larvae

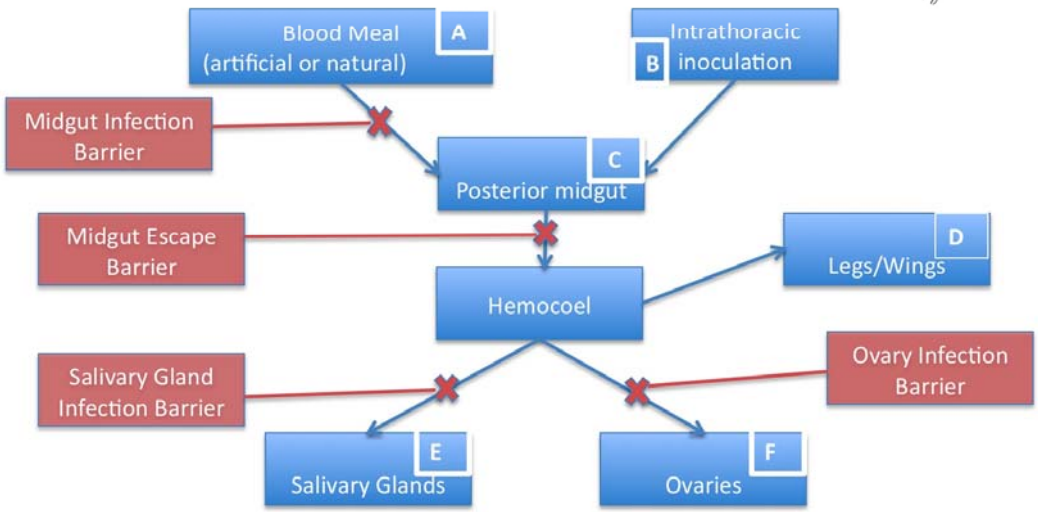
Pupae

Emerging adult

Colony of adults

The Mosquito as a Vector

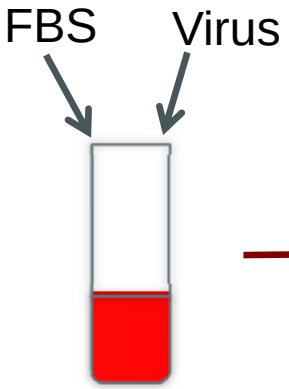




Experimental Methods

- Oral exposure through artificial blood meals
  - Analysis of bodies, legs, saliva
- Intrathoracic inoculation into hemocoel
  - Analysis of bodies, transmission
- Transmission
  - Saliva
  - Direct transmission to suckling mice

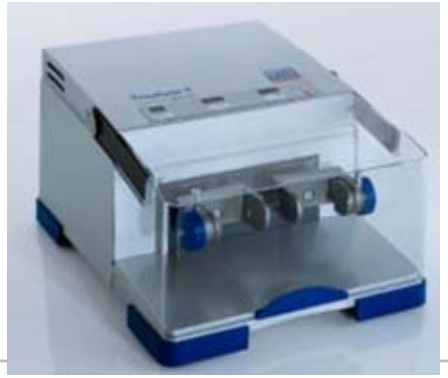
Oral Exposure



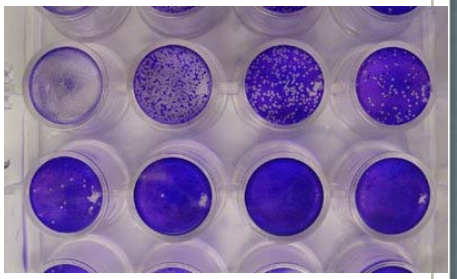
*

**

*



*



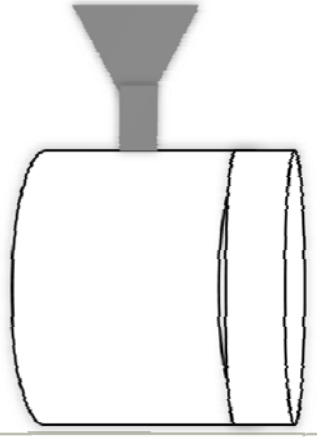
Intrathoracic Inoculation Exposure

+

**

Cold anesthetize

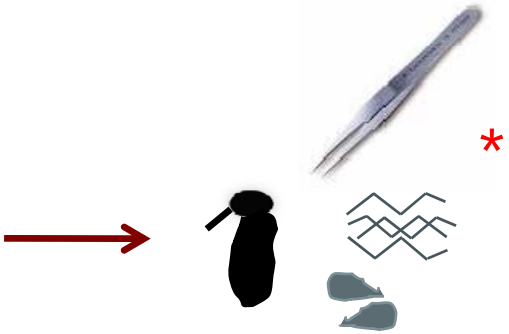
*



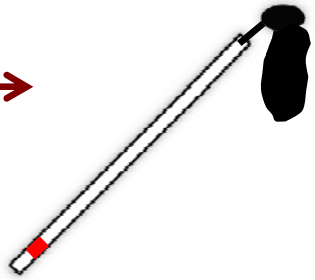
*

Transmission: Saliva

Cold anesthetize



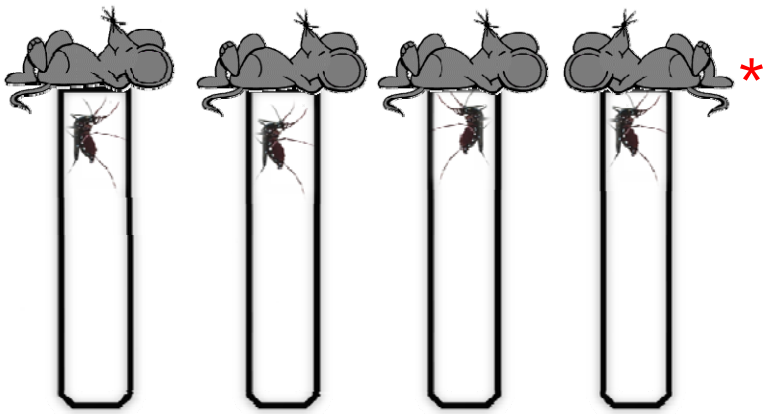
Dissect legs
and wings



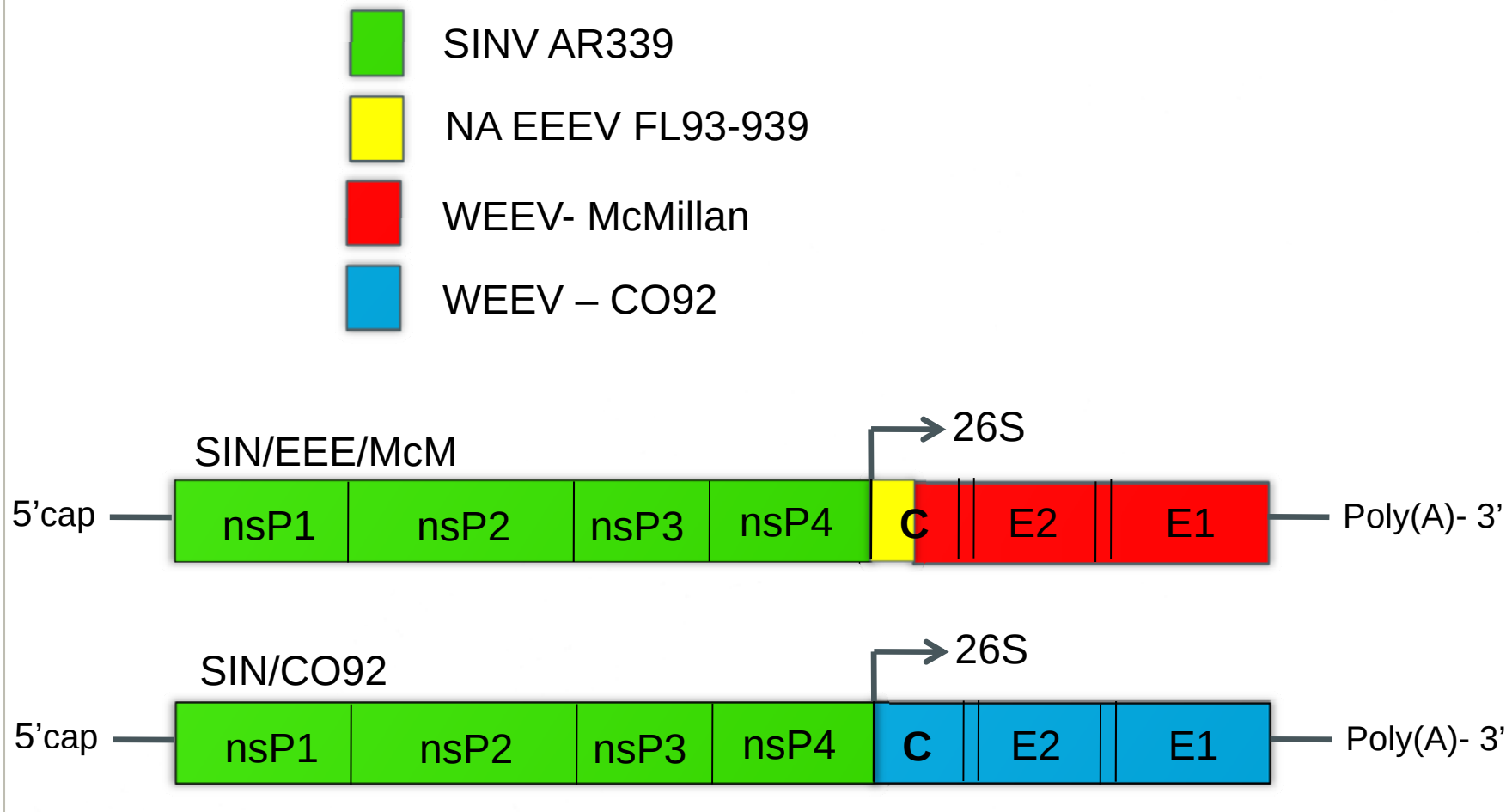
Break off end
of capillary

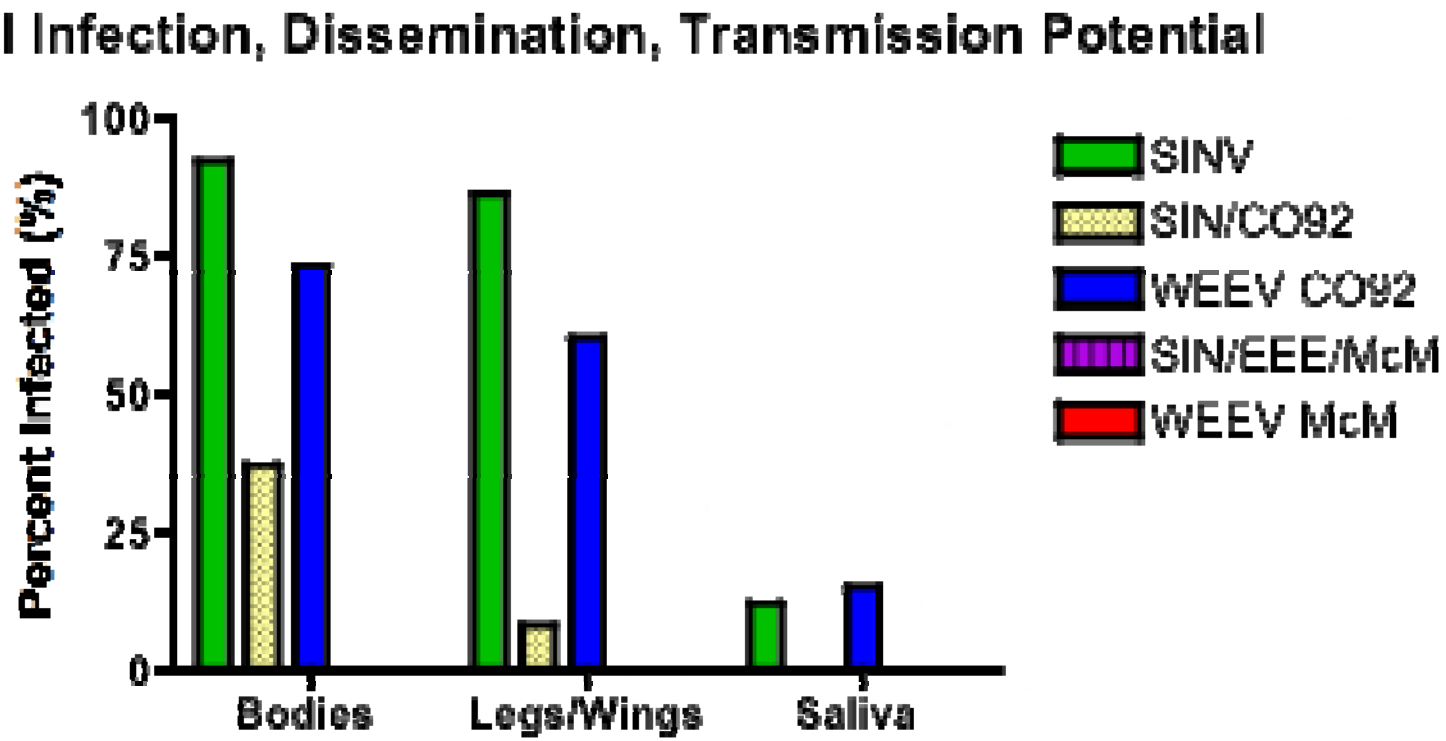
Transmission: Naïve Mice

Anesthetize and Sort



Live-Attenuated Chimeras





- Neither McMillan based vaccine infected *Cx. tarsalis*
- Salivary infection rates were low for wild type virus
  - Capillary method is not the most sensitive

Some remaining questions

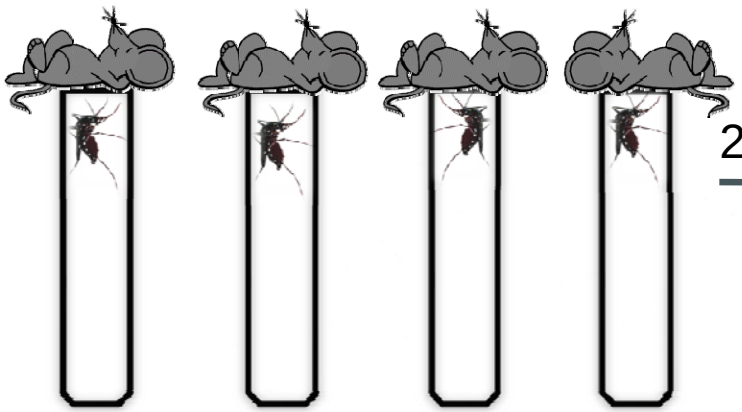
- Can McMillan based viruses replicate in *Cx. tarsalis* mosquitoes
- Can McMillan based viruses be transmitted (despite the lack of a natural route of infection)?
- What is the true transmission potential of these vaccine strains to naïve mice?

Can McMillan viruses replicate and transmit?

1. IT inoculate mosquitoes

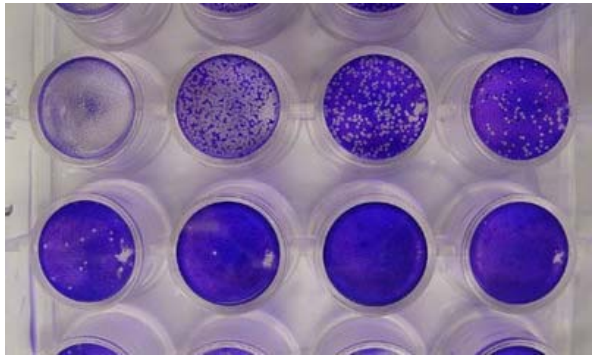
7 Day EIP

2. Cold Anesthetize



3. Feed individual mosquito on individual mouse

21 day hold



4. PRNTs

McMillan Transmission

SINV - 7/9 mosquitoes probed

- 7/7 transmitted
- 1 survived

SIN/EEE/McM – 9/10 probed

- 8/9 transmitted
- 2 survived

SINV

Mouse	Exposure	Date of Death	Survival Serostatus
1	probed	D4	-
2	probed	-	1:20
3	probed	D4	-
4	probed	D3	-
5	Not exposed	-	Neg
6	Not exposed	-	Neg
7	probed	D5	-
8	probed	D4	-
9	probed	D4	-

SINV/EEE/McM

Mouse	Exposure	Date of Death	Survival Serostatus
1	probed	-	1:40
2	probed	D4	-
3	Not exposed	-	Neg
4	probed	-	1:20
5	probed	-	Neg
6	probed	D4	-
7	probed	D3	-
8	probed	D4	-
9	probed	D4	-
10	probed	D5	-

McMillan Transmission Cont.

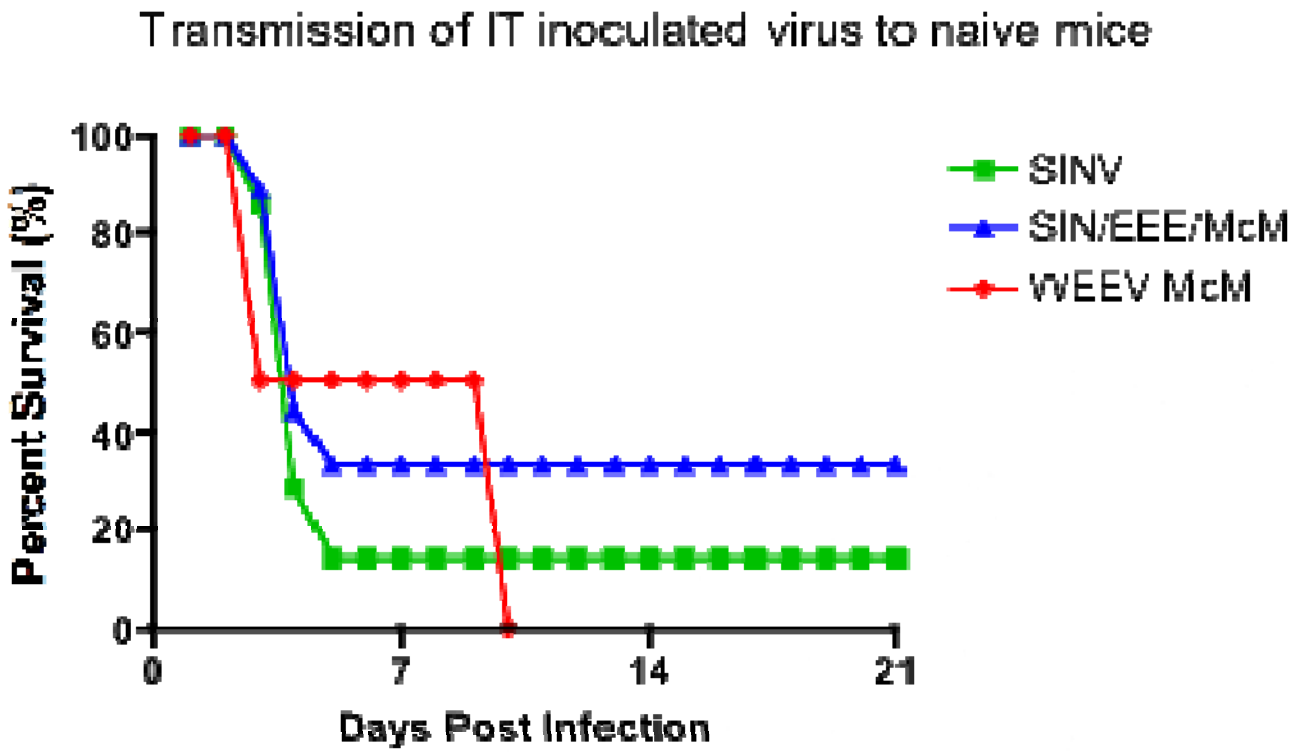
McM –3/7 probed

- 3/3 transmitted
- All 3 died
- Mother also infected, died

	McM		
Mouse	Exposure	Date of Death	Survival Serostatus
1	probed	D11*	Neg
2	Not exposed	D11*	-
3	probed	D3^	-
4	probed	D3^	-
5	Not exposed	-	Neg
6	Not exposed	-	Neg
7	Not exposed	-	Neg
Mother	consumed 3 and 4	D10*	1:20

* Killed by surrogate mother

^ Killed by original mother when showing signs of illness



Results: IT McMillan

- All intrathoracically inoculated mosquitoes were infected
- McMillan based viruses can be transmitted to naïve mice

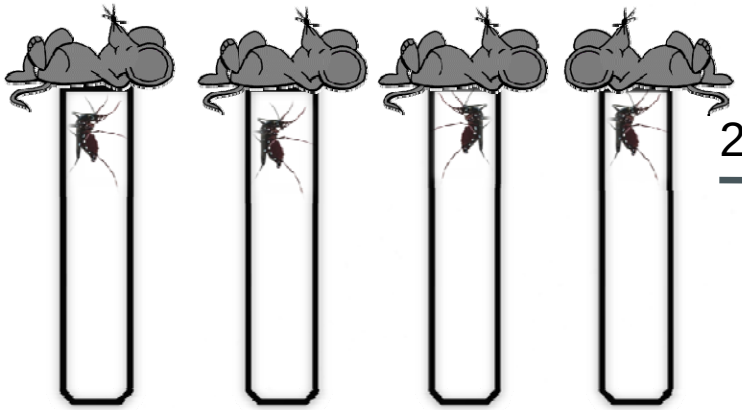
SIN/CO92

Can CO92 viruses be transmitted?

1. Feed mosquitoes

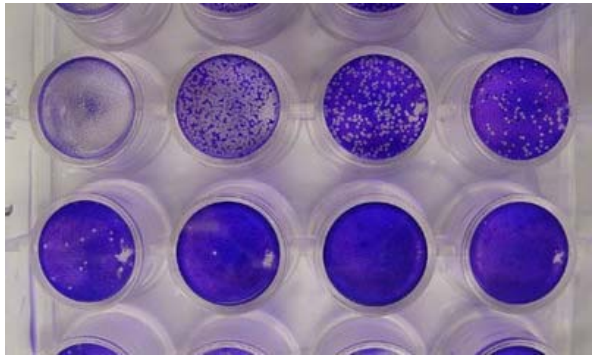
10 Day EIP

2. Cold Anesthesize

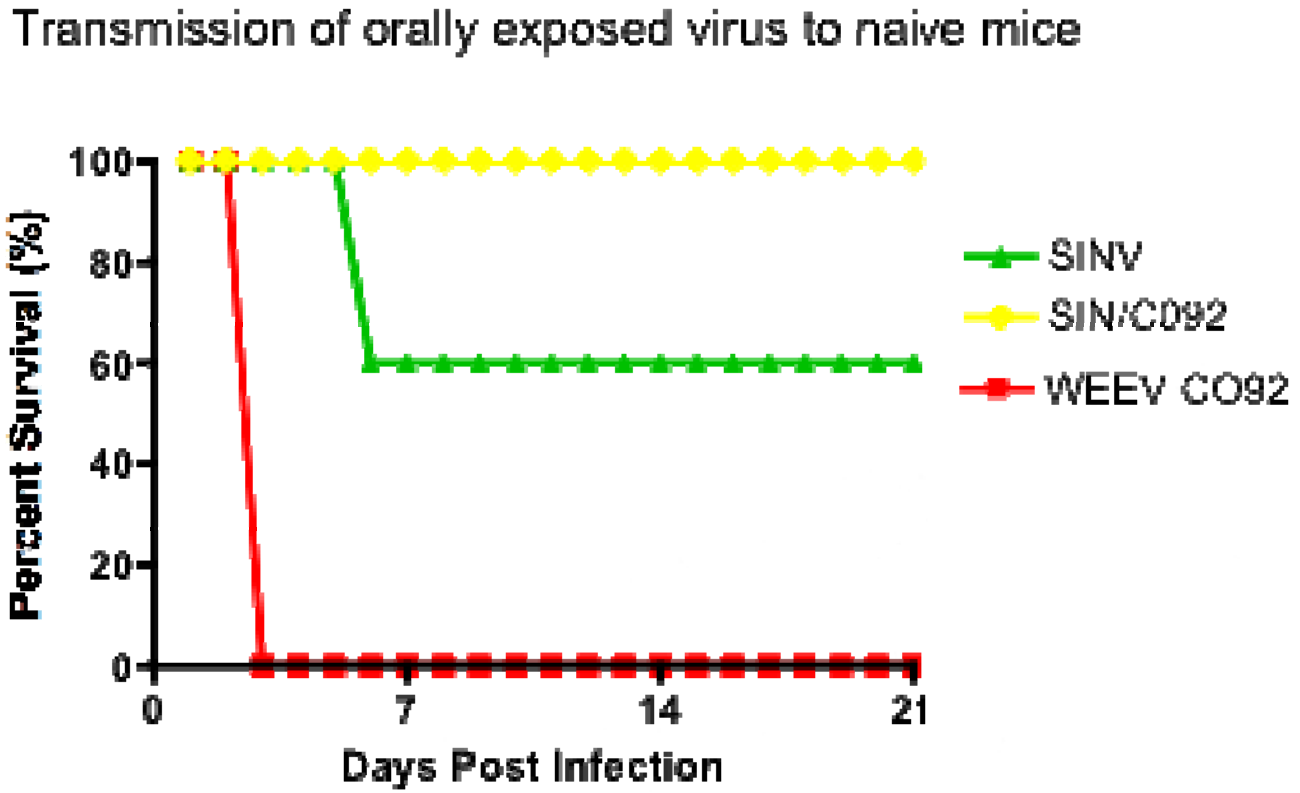


3. Feed individual mosquito on individual mouse

21 day hold



4. PRNTs



Results: CO92 Transmission

- We were unable to demonstrate transmission
  - The rates of dissemination were so low, we never had a disseminated mosquito feed on a naïve mice

Results Summary

- SIN/CO92 vaccine can orally infect and disseminate in *Cx. tarsalis* mosquitoes
  - We were unable to demonstrate transmission to naïve mice, nor were we able to find virus in the salivary glands
- McMillan based strains were unable to naturally infect the mosquito vector
  - However, when artificially infected, *Cx. tarsalis* was able to transmit SIN/EEE/McM and McM to naïve mice

Conclusions

- SINV, WEEV CO92, and SIN/CO92 are infectious for *Cx. tarsalis* following relatively large oral doses
  - SIN/CO92 can disseminate in mosquitoes, albeit at low rates
  - Virus detection in saliva by capillary method is very insensitive
- We conclude that transmission of SIN/CO92 is highly unlikely, due to the inability to demonstrate in a lab setting with high viral titers

Conclusions Cont.

- *Cx. tarsalis* mosquitoes are refractory to oral exposure with McMillan based strains
  - Likely due to unaccounted passages of stock
  - Determination of the mechanisms of this change in phenotype would be extremely valuable
- Both SIN/EEE/McM and WEEV McM can replicate and be transmitted following IT inoculation
  - Not too ecologically relevant

Final Conclusion

- Neither WEEV candidates are likely be acquired from a viremic host or transmitted by *Cx. tarsalis*
- Previous examination of a SIN/EEEV indicated similar results
- These findings indicate these candidates have superior environmental safety

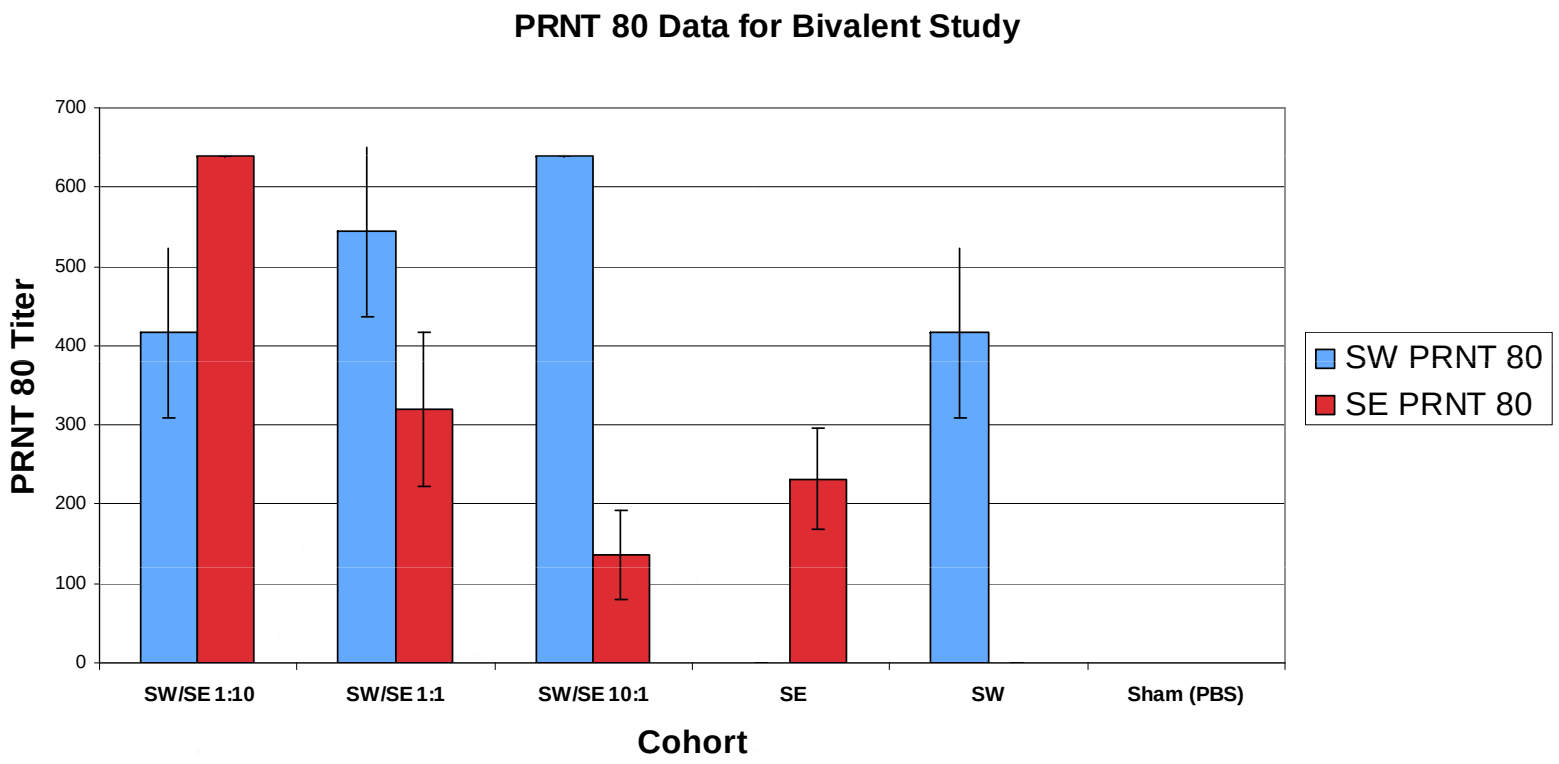
Future Directions

- Currently in the process of evaluating the genetic stability of the chimeric attenuation approach
- To put the SIN/EEE/McM, SIN/EEE, and the SIN/VEEV together and evaluate protection and safety in mice
- IRES elements the future of arboviral vaccine safety?
  - Internal ribosomal entry site (from Picornoviruses) inhibit viral replication in insects

Acknowledgements

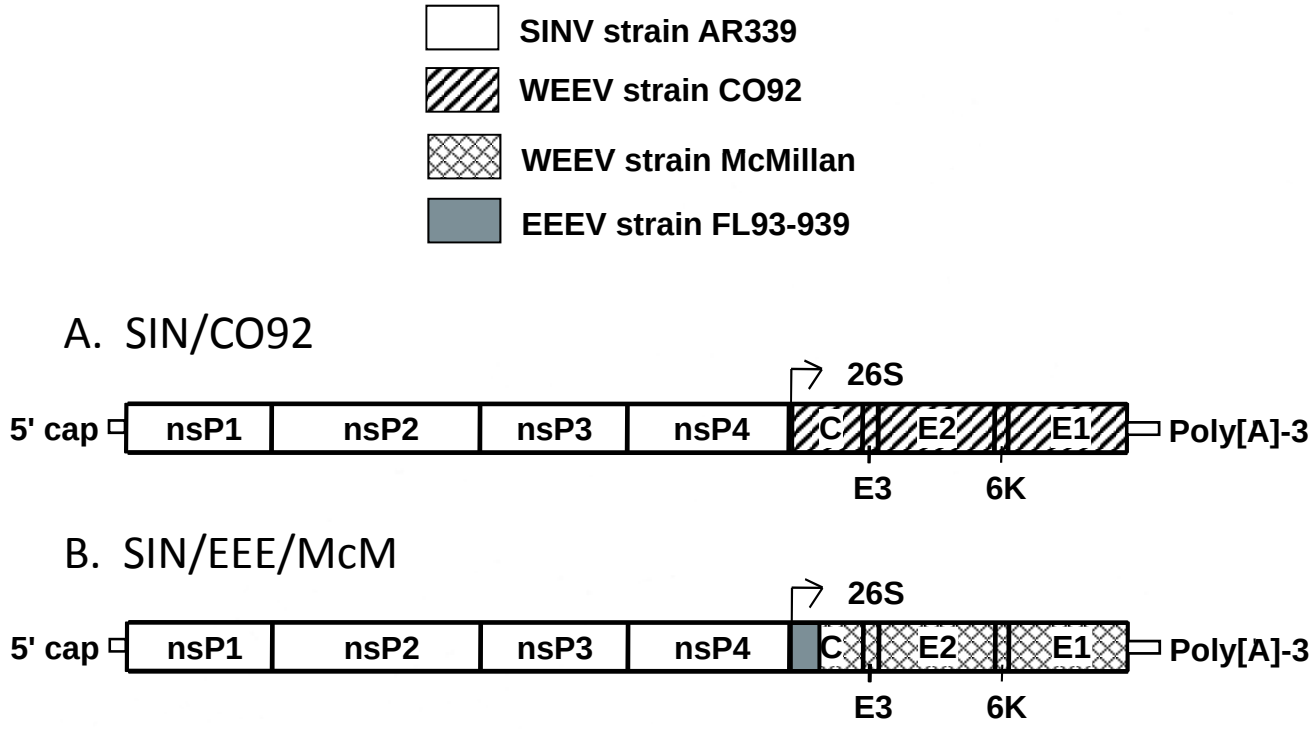
- Weaver lab members
  - Scott Weaver
  - Paige Adams
  - Nicole Arrigo
  - Naomi Forrester
- Higgs Lab
  - Steve Higgs
  - Jing Huang
- Frolov Lab
- UC Davis
  - Bill Reisen

Does it hold it's own as a bivalent vaccine with EEEV?



Dr. Eryu Wang and Kenney Plante

Fig. 1


SINV strain AR339
WEEV strain CO92
WEEV strain McMillan
EEEV strain FL93-939
A. SIN/CO92
26S
5' cap
nsP1
nsP2
nsP3
nsP4
C
E2
E1
Poly[A]-3
E3
6K
B. SIN/EEE/McM
26S
5' cap
nsP1
nsP2
nsP3
nsP4
C
E2
E1
Poly[A]-3
E3
6K

WEEV History

- WEE was recognized during a 1930 outbreak in San Joaquin Valley, CA
  - Virus was isolated by intraocular inoculation into a healthy horse (Meyer et al. 1931)
- 1933 mosquito-borne transmission shown between guinea pigs and *Aedes aegypti* (Kesler 1933)
- By 1935 WEE had reached Canada (Cameron 1942)
- 1941 WEE isolated from *Culex tarsalis,* serologically incriminated in birds in Yakima Valley, WA (Hammon et al. 1941, 1942, 1943)
- Experimental infection in the lab established *Cx. tarsalis* as a competent vector (Hammon et al. 1943)

Disease in Humans cont.

- Typical onset of illness is characterized by sudden fever, chills, headache, nausea, vomiting, and occasionally respiratory symptoms
- If CNS symptoms develop, that occurs between one to seven days and is characterized by lethargy, drowsiness, stiff neck, photophobia, vertigo, irritabilty, generalized convulsions and tremors (Kokernot et al. 1953; Medovy 1943)

Alphavirus Background

Alphaviral encephalitides

Antigenic complex	*Species*	*Antigenic subtype*	*Antigenic variety*	*Equine clinical syndrome*	*Distribution*
Eastern equine encephalitis (EEE)	EEE		North American	Encephalitis	North America, Caribbean
			South American	Encephalitis	South, Central America
Venezuelan equine encephalitis (VEE)	VEE	(I) VEE	AB	Encephalitis	South, Central, North America
			C	Encephalitis	South, Central America
			D	None or mild febrile illness	South, Central America
			E	None or mild febrile illness	Central America
			F	None reported	Brazil
		(II) Everglades		None or mild febrile illness	Florida, USA
		(III) Mucambo	A (Mucambo)	None or mild febrile illness	Brazil, Trinidad
			B (Tonate)	None reported	French Guiana
			B (Bijou Bridge)	None reported	western North America
			C (71D-1252)	None reported	Peru
		(IV) Pixuna		None or mild febrile illness	Brazil
		(V) Cabassou		None reported	French Guiana
		(VI) (AG80–663)		None reported	Argentina
Western equine encephalitis (WEE)	WEE		Several	Encephalitis	North, South America
	Highlands J			Rare encephalitis	eastern North America
	Ft. Morgan			None reported	western North America
	Buggy Creek			None reported	Oklahoma, USA
	Y62-33			None reported	Russia
	Sindbis	(I) Sindbis		None reported	Africa, Asia, Europe, Australia
		(II) Babanki	None reported	Africa	
		(III) Ockelbo		None reported	Europe
		(IV) Whataroa		None reported	New Zealand
		(V) Kyzylagach	None reported	Azerbaijan	
	Aura			None reported	South America

EPIDEMIOLOGY OF VETERINARY ENCEPHALITIDES

Adapted from Weaver et al. 1999