

# **CURRENT STATUS OF WEST NILE VACCINE DEVELOPMENT**

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# Major flavivirus diseases

- Dengue
- Japanese encephalitis
- Tick-borne encephalitis
- West Nile
- Yellow fever

# Current flavivirus vaccines

## Live attenuated

- Israel turkey meningoencephalomyelitis (V)
- Japanese encephalitis (B)
- Wesselsbron (V)
- Yellow fever (H)

## Inactivated

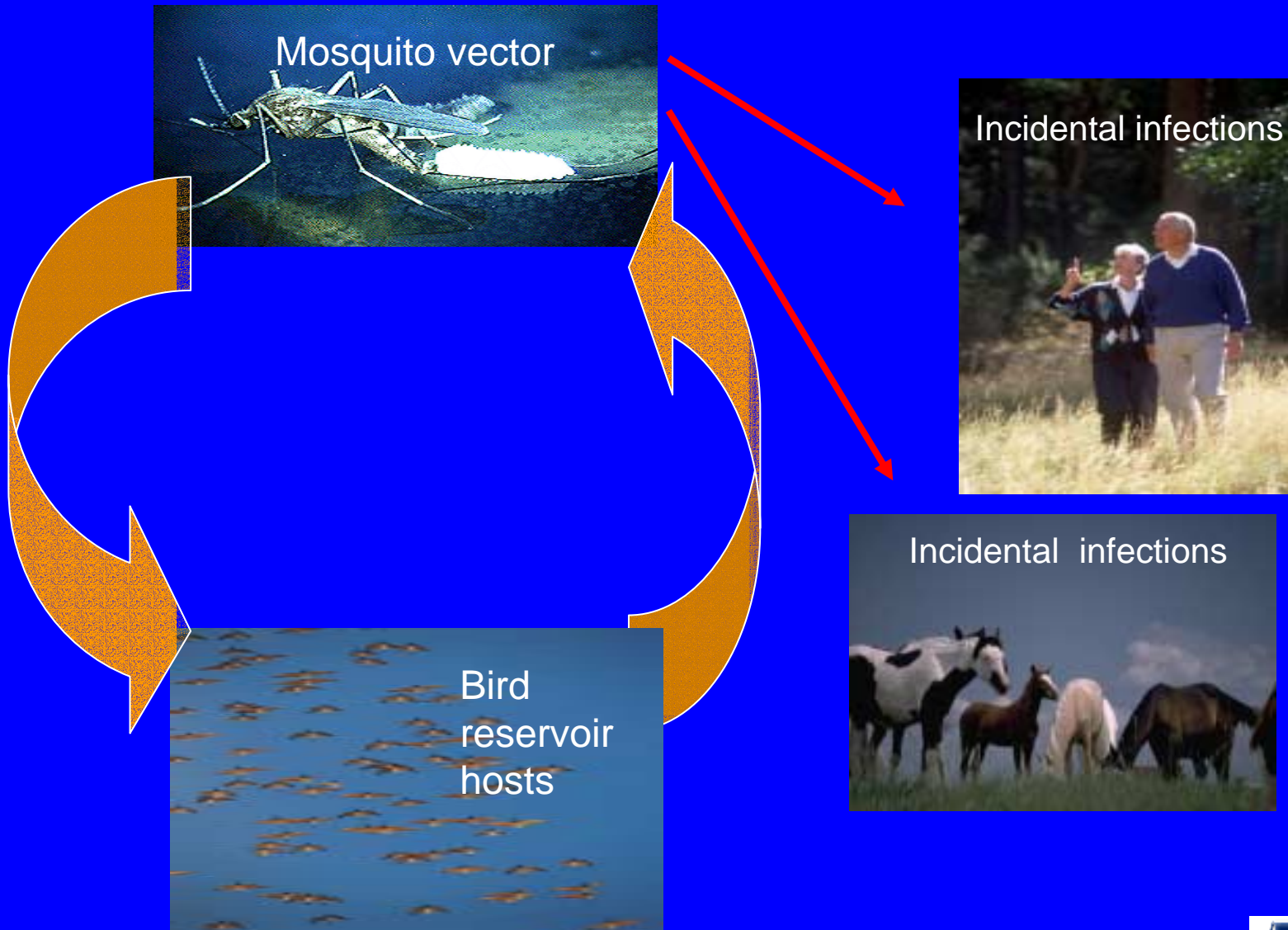
- Japanese encephalitis (H)
- Kyasansur Forest disease (H)
- West Nile (V)
- Tick-borne encephalitis (H)

H = human, V = veterinary, B = both

# West Nile vaccines

- Human vaccine.
- Veterinary vaccine.

# West Nile Virus Transmission Cycle



# Potential hosts of West Nile virus

- At least 225 species of birds
- At least 49 species of mosquito
- At least 28 species of mammals, inc. cats, dogs, sheep, llama, wolf, goats, squirrels, skunks, etc...
- Alligators.....

# West Nile virus

- Family: *Flaviviridae*
- Genus: *Flavivirus*
- Japanese encephalitis group

*Cacipacore virus*

*Koutango virus*

***Japanese encephalitis virus***

*Murray Valley encephalitis virus*  
(Alfuy virus)

***St. Louis encephalitis virus***

*Usutu virus*

***West Nile virus***

(Kunjin virus)

*Yaounde virus*

# Approaches to developing vaccines

- Inactivated virus
- Live attenuated
- Recombinant poxviruses
- Infectious clone-derived live attenuated
- Chimeric viruses
- Subunit
  - Yeast
  - *E. coli*
  - Baculovirus
- DNA/RNA



# JE vaccine does not induce neutralizing antibodies against WNV

- Kanesa-Thasan et al AJTMH 66: 115-116, 2002.
- Volunteers immunized with inactivated JE vaccine and bled after vaccination.
- Sera did not contain antibodies that neutralized WNV.
- Inactivated JE vaccine will not protect against WNV?

# West Nile vaccine in Israel

- Investigated Israel Turkey meningoencephalomyelitis virus (ITMV) as vaccine in 3 week old geese.
- Live attenuated chick embryo adapted vaccine developed in 1975 ( $10^3$ EID<sub>50</sub>/0.5ml).
- No NT ab but 92% protection in geese.
- Why does ITME vaccine protect against WNV challenge?

# Inactivated vaccine protects geese against WN challenge

- Studies in Israel
- Prepared mouse brain formalin-inactivated WN vaccine based on goose (NY-like) strain.
- Geese challenged with WN virus by ic route
- Killed WN virus gave 52-80% protection

# Veterinary vaccine I

- Available in some areas during 2001
- Manufactured by Fort Dodge Animal Health.
- Formalin-inactivated, Killed Vaccine; two doses, 1ml, IM, 3 weeks apart
- \$20-30 per dose
- Booster doses required annually
- Effectiveness?

# Veterinary vaccine II

- Ng et al Dev Biol Stand 114: 221-227, 2003
- Amount of antigen in dose not stated.
- NT abs higher than WNV DNA vaccine in horses at day 14 post second dose.
- 17/19 horses NT ab positive (1:5) @ 1 year post vaccination.
- 1/19 vaccinated horses had viremia following challenge → 94% preventable fraction.

# Veterinary vaccine III

- Nusbaum et al Avian Dis 47: 750-752, 2003
- Immunized Chilean flamingos and red tailed hawks IM with 0.2ml commercial inactivated vaccine. None of 13 birds tested had detectable ab to WNV.
- Tesh et al EID 8: 1392-97, 2002
- 8/9 hamsters had HI & CF ab; 5/9 had low PRNT (1:10-40);
- 2/9 hamsters had viremia ( $\leq 20,000$  pfu/ml) post challenge with 10,000 pfu WNV

# Inactivated vaccine for humans?

- Baxter-Immuno have inactivated tick-borne encephalitis vaccine marketed in Europe.
- TBE formalin-inactivated cell culture
  - 2 doses, 2-13 weeks apart; booster 1 year,
  - re-boost every 3-5 years
  - >95% seroconversion after two doses
- Using same technology for inactivated WN vaccine?

# West Nile DNA vaccine I

- Davis et al. J. Virol. 75, 4040-47, 2001
- prM & E genes from WN –NY
- JEV transmembrane signal sequence
- Immunize: one dose by IM route
- Mice – 3-week-old female ICR; challenge ip 1000LD50 at 6 weeks post-immunization or via mosquito → 100% protection.
- Equines – 4 of various ages; challenge via mosquito → seroconversion & no viremias



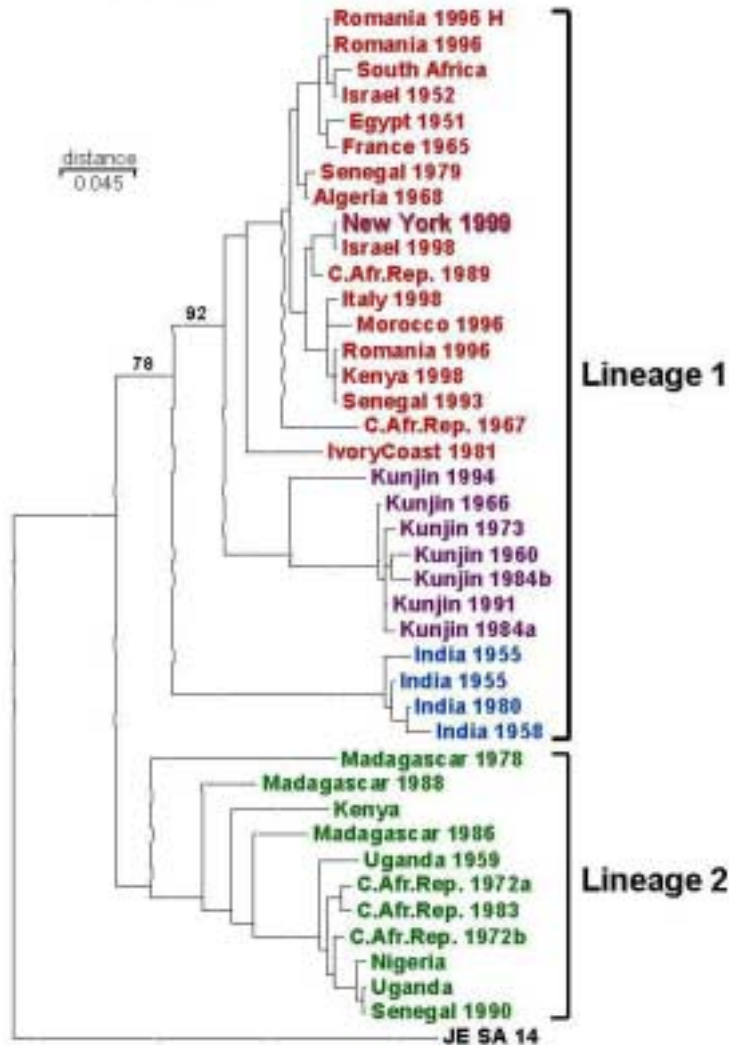
# West Nile DNA vaccine II

- Yang et al. JID 184:809-16, 2001
- Synthetic C gene from WN –NY
- Used human Ig secretory leader signal
- Immunize: one dose by im route
- Mice – 6-8-week-old female Balb/c
- Antigen specific humoral and cellular immune responses
- Antigen specific Th1 and CTL response
- No challenge studies.

# WN DNA vaccine III

- Turell et al. EID 9: 1077-81,2003
- 0.5mg DNA vaccine (Davis et al vaccine) by oral or IM routes in fish crows (*Corvus ossifragus*).
- 9/9 IM vaccine and 4/8 oral vaccine immunized birds survived WNV challenge.
- No NT abs prior to challenge and no sterile immunity following challenge.

## Phylogenetic Tree Based on Envelope Glycoprotein Sequence Data



MEGA, distance tree, Kimura 2-parameter, neighbor-joining

Lanciotti et al. 1999.  
Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern U.S.  
[Science 286:2333-337.]

# Kunjin virus as a vaccine?

- Hall et al PNAS 100:10460-64, 2003
- KUN virus in plasmid.
- 4-5 week old Balb/c mice immunized i.m. with KUN plasmid
- Viremia very low.
- PRNT: 1:10-20
- 0.1-1ug KUN plasmid DNA protected mice from challenge
- 1ug protected mice against ic or ip challenge  
20pfu WNV

# Canarypox-vectored vaccines

- Veterinary vaccines; used in Europe
- KANAPOX™ derived from ALVAC
- Applicable to mouse, ferret, dog, cat, horse, pig & primates
- WNV prM/E vectored vaccine
- Antivector immunity does not interfere with booster immunizations.
- Can be combined with live & inactivated vaccines as immunogens.

# Recombinant WN-E protein

- Wang et al. J Immun 167: 5273-5277, 2001
- Thioredoxin 100%E and mbp-80%E
- Immunize C3H/HeN mice with 20ug rE-fusion protein
- Serum PRNT = 1280
- Mice protected from 10LD<sub>50</sub> challenge
- Limited passive protection
- Other recombinant proteins being evaluated by other laboratories

# ChimeriVax™ Technology Platform

*Chimeric live attenuated vaccines utilizing yellow fever 17D as a vector for envelope genes of other flaviviruses*

ChimeriVax™-JE (Japanese encephalitis)

ChimeriVax™-DEN (Dengue)

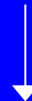
ChimeriVax™-WN (West Nile)

# Derivation of Attenuated Yellow Fever 17D Virus Strain

Asibi Virus



176 passes in chick  
embryo tissue



17D Attenuated  
Variant

(17D-204 p233-239)

(17DD p286-288)



# ChimeriVax™

- Promising vector for novel flavivirus vaccines
- Less neurovirulent than parental YF 17D
- Infectivity (viremia) similar to YF 17D
- Neutralizing antibody responses similar to YF 17D
- Protects against severe challenge (e.g. JEV by IC route in monkeys)
- Not infectious for mosquitoes
- Well-tolerated and immunogenic in humans (JE)

# ChimeriVax<sup>TM</sup>-WN

Used WN-NY #383-99 for prM & E genes

## Human vaccine

- WN-F<sub>107</sub>-V<sub>316</sub>-R<sub>440</sub>
- ChimeriVax-WN 10<sup>4</sup> pfu sc → protects 12/12 monkeys against ic challenge of 10<sup>5</sup> pfu WN-NY99 → no viremia/clinical signs

## Veterinary vaccine

- WN wildtype
- Johnson et al Med Vet Ent 17:235-43, 2003
- ChimeriVax-WN replication restricted in mosquitoes → *Cx. tritaeniorhynchus*, *Cx. nigipalpus*, *Cx. quinquefasciatus*, *Ae. aegypti* and *Ae. albopictus*.

# ChimeriVax<sup>TM</sup>-WN is not efficacious in Fish Crows (*Corvus ossifragus*)

- Langevin et al AJTMH 69: 78-80, 2003
- ChimeriVax-WN failed to prevent viremia (8/8) in immunized birds.
- 2/8 birds succumbed to WNV challenge
- Yellow fever virus does not replicate in birds.

# DEN4/WN chimeras

- Pletnev et al. PNAS 99: 3036-41, 2002; Pletnev et al. Virol 314:190-95, 2003
- Used DEN4 backbone with WN prM/E genes.
- WN neurovirulence reduced 20,000-fold in 3-day-old mice
- WN neuroinvasiveness reduced >10,000-fold in 3 week old mice
- IP immunization with 1000 ffu → 1:1200 PRNT
- ≥90% mice resistant to 100LD<sub>50</sub> challenge
- WN/DEN4 chimera gave 100-fold reduction in viremia compared to WN or DEN4 in rhesus monkeys
- Prevented viremia in WN challenged monkeys