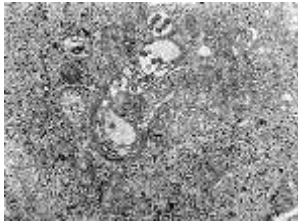


17^{ème} Colloque sur le Contrôle Epidémiologique des Maladies Infectieuses

15 et 16 mars 2012 – Institut Pasteur - Paris

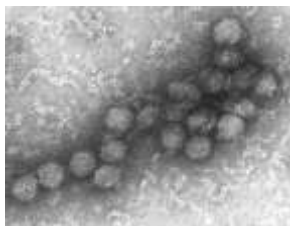
Pathogenic arboviruses: RVFV and WNV



| Virus | Classification: family genus | Vector | Animals affected | Disease (SFI ¹ , HF ² , E ³) | Endemic presence | OIE* listed |
|-------------------------|---|-------------------------------|---|---|---|----------------|
| Rift Valley fever virus | <i>Bunyaviridae</i> <i>Phlebovirus</i> | Mosquito (<i>Aedes</i> spp.) | Humans, sheep, goats, camels | SFI/HF/abortion | Africa | Yes |
| West Nile virus | <i>Flaviviridae</i> <i>Flavivirus</i> | Mosquito (<i>Culex</i> spp.) | humans, cattle, horse, avian + many other | E | Africa, Eurasia, Americas Australia | Yes |

¹ SFI: systemic Febrile Illness; ² HF: haemorrhagic fever; ³ E: encephalitis.

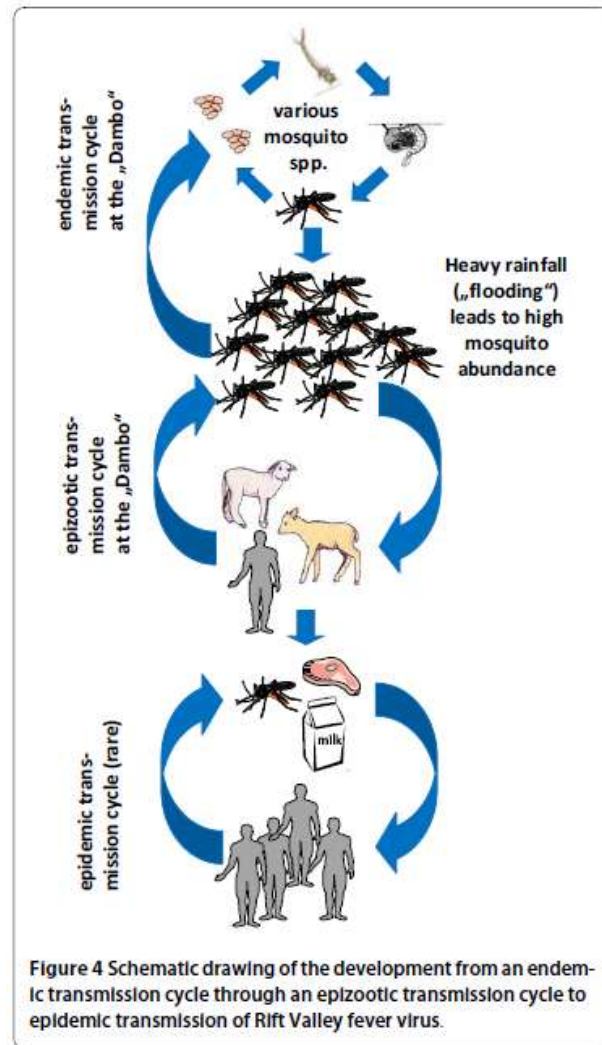
*Office International des Epizooties (World Organisation for Animal Health).



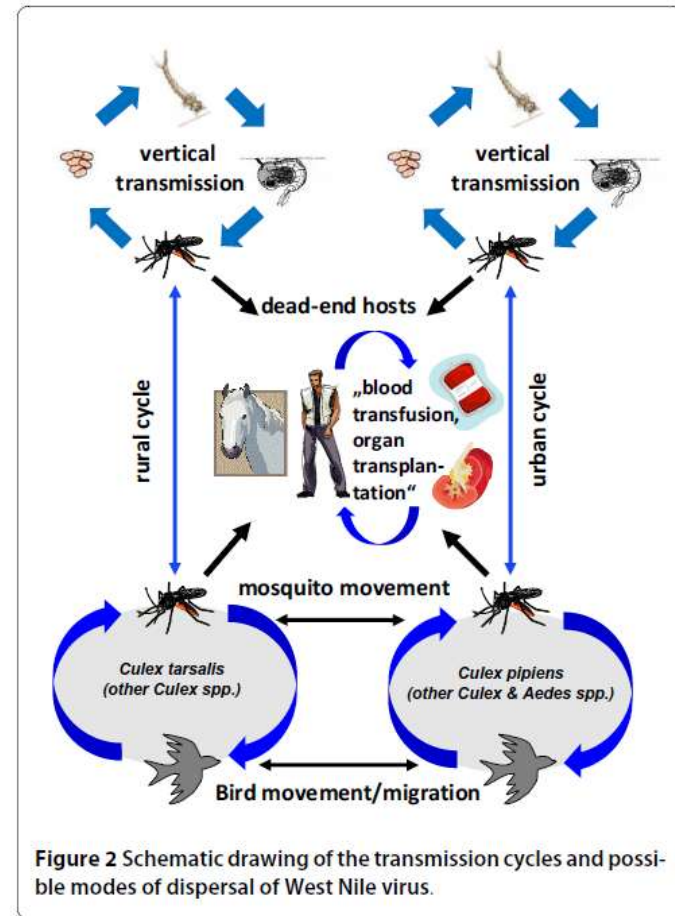
Adapted from N. Johnson et al., JBB, 2012
Rapid molecular detection methods for
« Arboviruses of livestock of importance to
Northern Europe »

Rift Valley Fever Virus and West Nile Fever Virus : transmission

RVFV



WNV

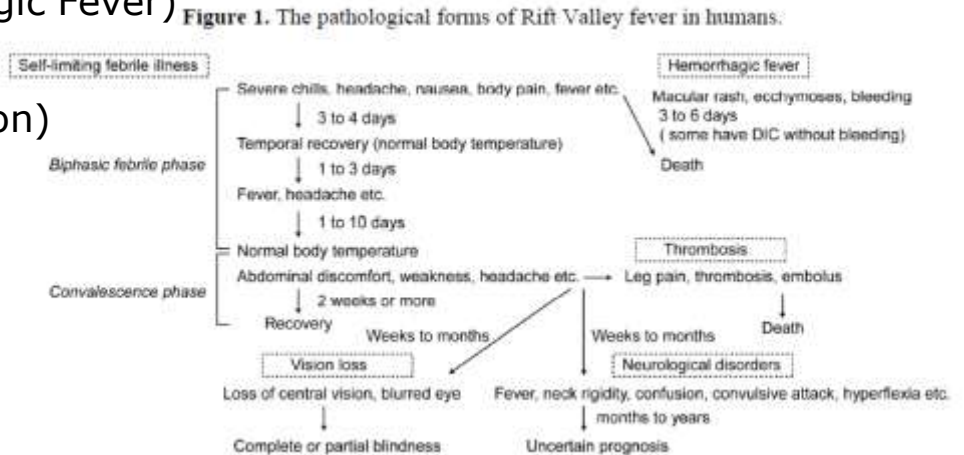


Pfeffer and Dobler *Parasites & Vectors* 2010, **3**:35
<http://www.parasitesandvectors.com/content/3/1/35>

Rift Valley Fever Virus and West Nile Fever Virus: clinical symptoms in humans

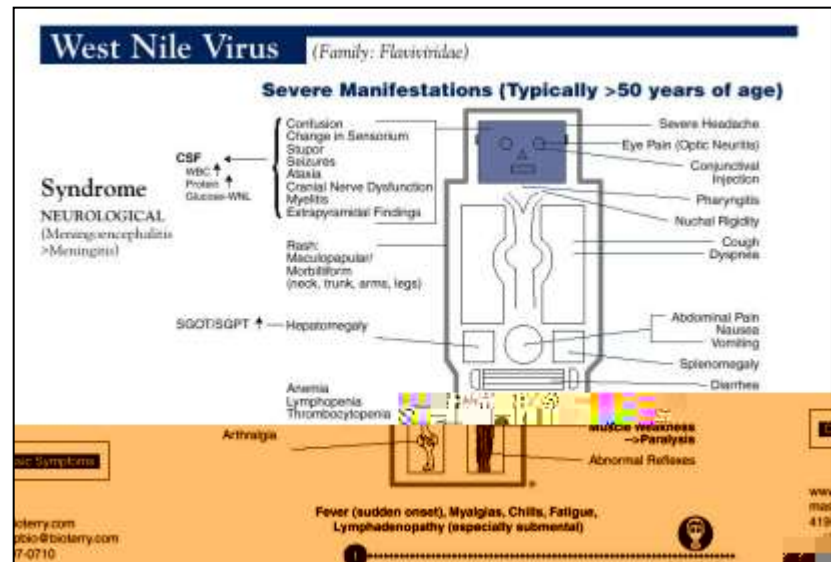
RVFV: (Systemic Febrile Illness, Haemorrhagic Fever)

- Asymptomatic (vast majority of cases)
- Influenza-like syndrome (small proportion)
- Severe RVF disease
 - Wide range of clinical signs
 - Hepatitis
 - Retinitis
 - Encephalitis
 - Hemorrhagic disease



WNV: Encephalitis

- Asymptomatic (approx. 75%)
- West Nile fever (approx. 25%)
- Neuroinvasive disease (approx. 1/140)
 - Encephalitis
 - Meningitis
 - Flaccid paralysis

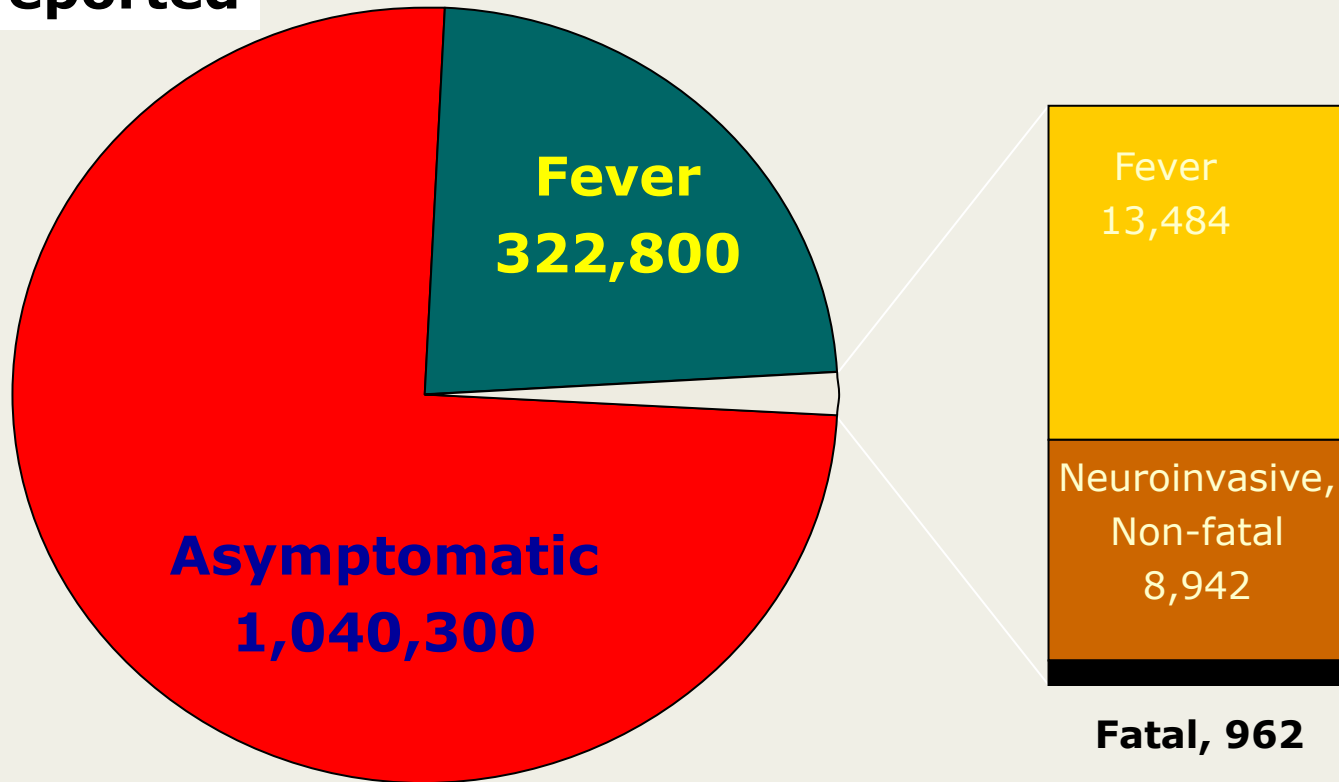


West Nile Disease: The hidden Epidemics

Human WNV Infections in the US, 1999-2006 (n=1.4 million)

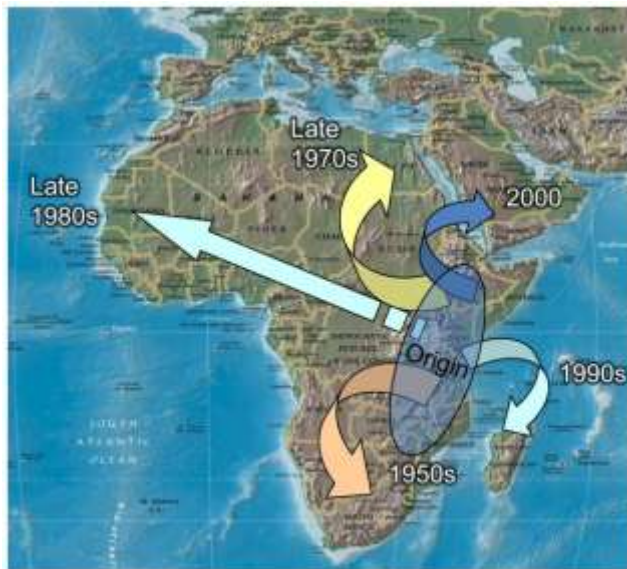
Not reported

Reported



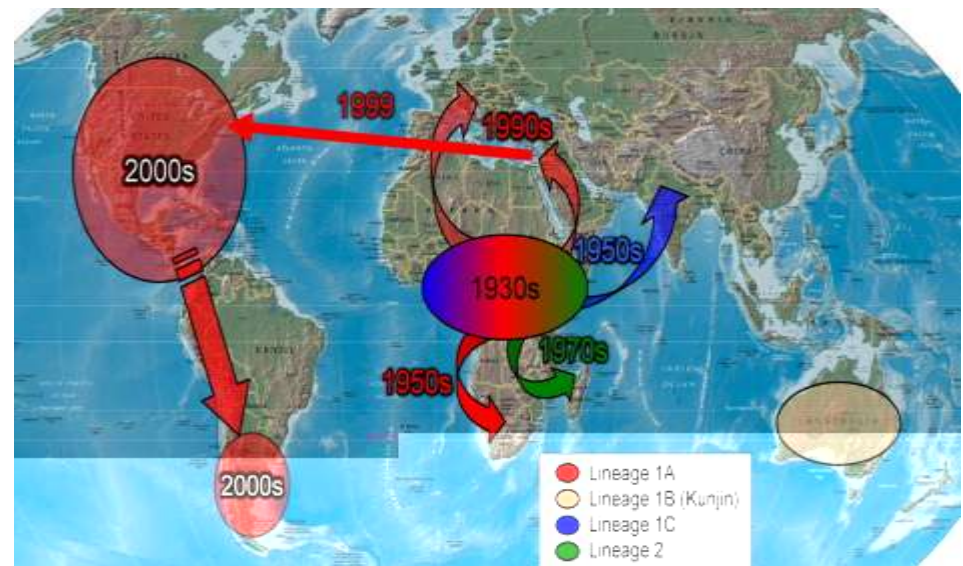
Courtesy L. Petersen, CDC

RVFV



Temporal patterns of dispersal by RVFV in Africa (Bird et al., 2003). Dates indicate the earliest detection and possible establishment of virus in each area.

WNV



Probable temporal sequence and dispersal routes of WNV from its proposed center of origin in sub-Saharan Africa (Powers et al., 2000) (Lanciotti et al., 2002)

A matter of climate change?

Location: Euro-Mediterranean Event: West Nile virus

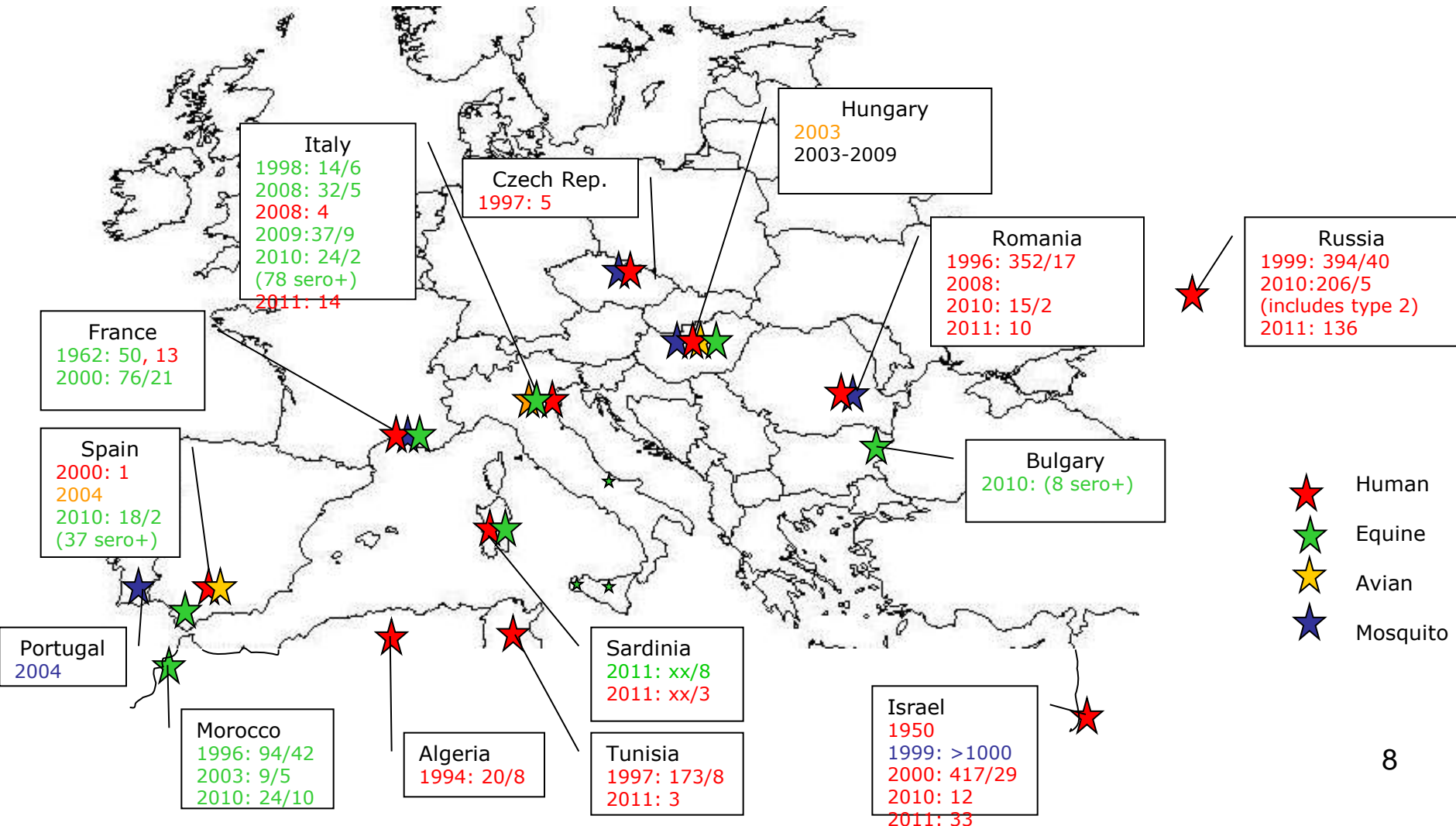
Map 4. Outline of main migratory ways and West Nile outbreaks recently notified (map from InVS/DIT)

Episouth, 2010

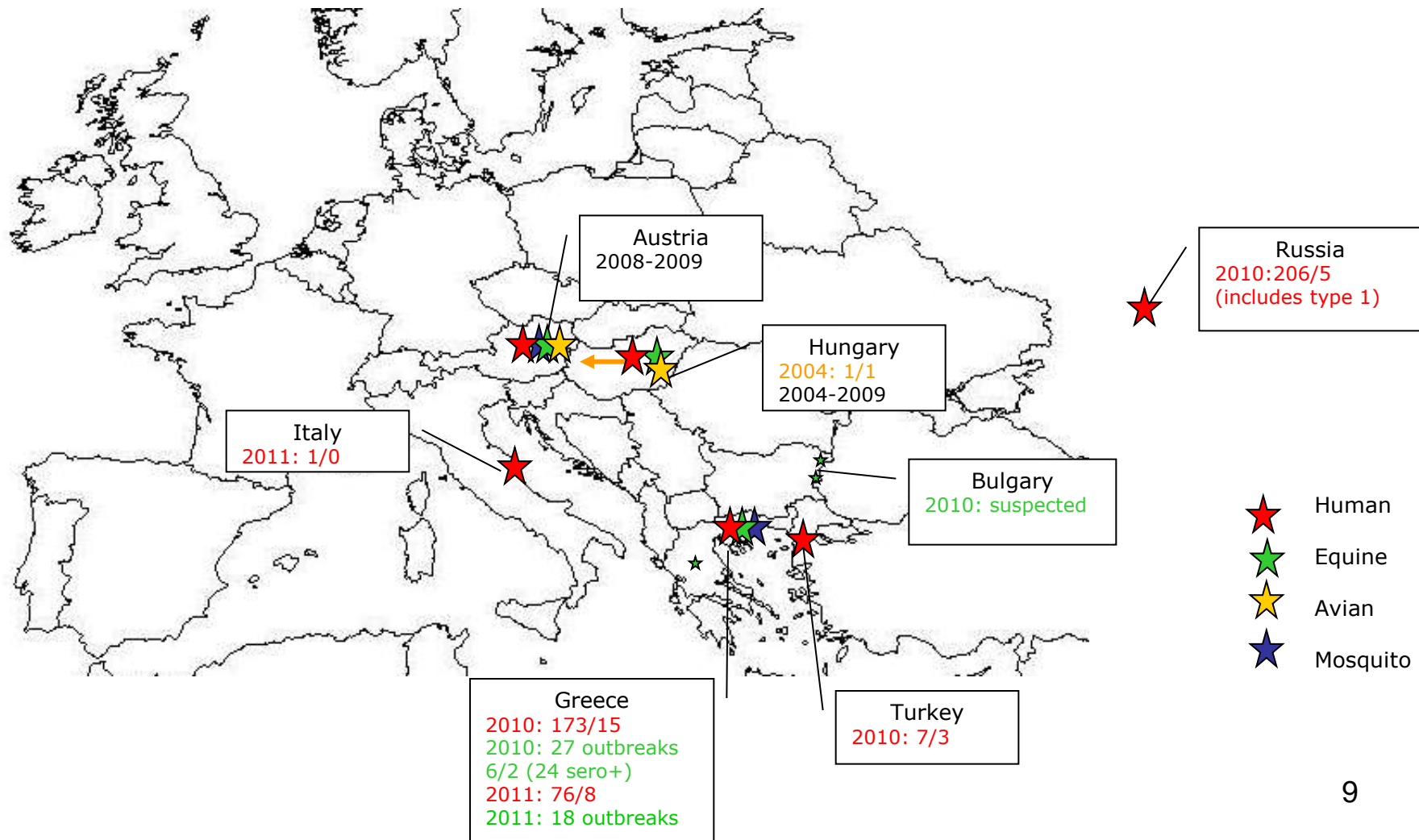


- No... Most likely viraemic migratory birds brought the virus from Africa to Europe, or infected ticks on migratory birds.
- BUT: Higher temperatures in spring, summer and autumn allow mosquitoes more reproduction cycles and thus contribute significantly to the establishment of a new infection in an area and facilitate the spread of the infection
- Also, the competent vectors for virus propagation and transmission have already been present in Europe (*Culex sp.*) and new vectors spread thorough Europe

Epidemiology – WNV **lineage 1**, reported cases of WNND/death at peak by year and by species



Epidemiology – WNV **lineage 2**, reported cases of WNND/death at peak by year and by species



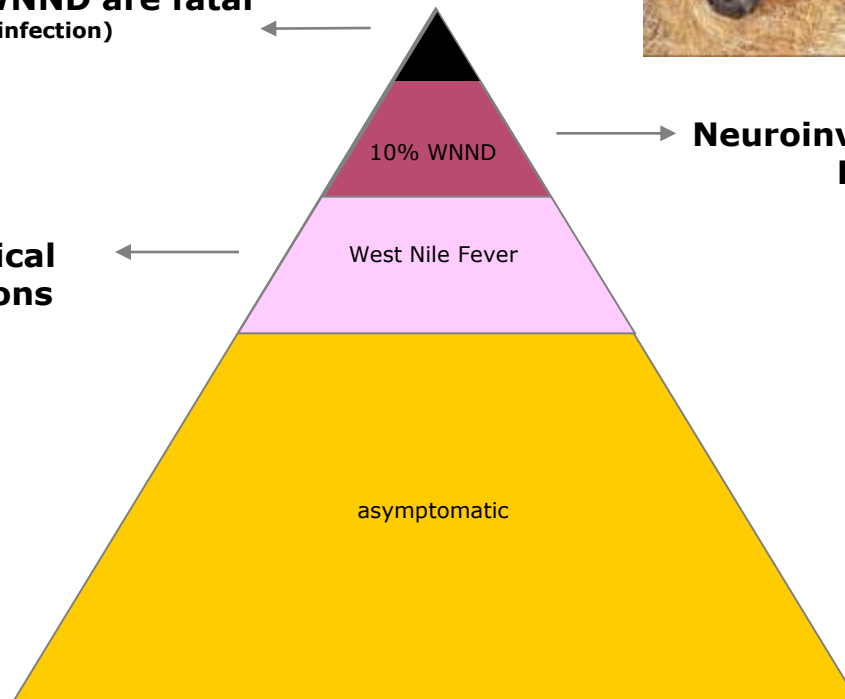
West Nile Virus: Disease in horses



10-60% of WNND are fatal
(0.1-0.6% of total infection)

Flu-like clinical manifestations

**Neuroinvasive Disease (WNND)=
Encephalomyelitis (WNE)**



J. Castillo-Olivares, J. Wood, 2004

West Nile disease: Symptoms in horses

- **Weakness (94%)**
- **Ataxia (72%)**
- **Abnormal Mentation (67%)**
- **Increased Body Temperature (65%)**
- **Fasciculation (61%)**
- **Anorexia (57%)**
- **Cranial Nerve Deficits (44%)**
- **Teeth Grinding (20%)**



Courtesy Dr. G. Dauphin

30% mortality in the US among confirmed clinical cases

- Veterinary vaccines: African Market

| Name of the vaccine | MA | Type | Characteristics | Secondary effects | Advantages | Drawbacks |
|--|--------------------------------|---------------------------|--|---------------------------------------|--|---|
| Live RVF (Smithburn strain, Ouganda) OBP | Africa | Live attenuated /Non DIVA | Long-life immunization One single shoot | Abortion Teratology in the fetuses | High immunogenicity | Potential risk of reversion or rest of virulence Use only in non- pregnant animals |
| Inactivated RVF (South Africa field strain) OBP | Africa | Killed /Non DIVA | Booster needed 3 to 6 months after initial vaccination Annual vaccination | No side-effect | Efficient after several boosts Safe, including in pregnant animals | Lower efficacy Short term immunity |
| Clone 13 (Central African Republic, human isolate, deletion of 70% in NSs) OBP / Institut Pasteur | Some countries in Africa (RSA) | Live attenuated /Non DIVA | | No side-effect | Safe, and efficient including in pregnant animals Inability to revert | |

West Nile Fever Virus : vaccines

- Veterinary vaccines: European market

| Name of the vaccine | MA | Type | Characteristics | Secondary effects | Advantages | Drawbacks |
|---|------|---|---|-------------------|--|--|
| Duvaxyn West Nile Virus (FDAH-Pfizer)* | EU | Formalin inactivated and adjuvanted whole virus | 2 shoots the first year Annual vaccination | No side effect | Reduction in viremia | Immunity: 4 weeks after the second injection |
| Proteq West Nile (Merial)** | EU | Live Canarypox vectored vCP2017 | 2 shoots the first year Annual vaccination Safe, including in pregnant animals True DIVA potential | No side effect | Immunity: 4 weeks after the first injection Reduction in viremia and clinical signs Cross-efficacy L1-L2 | |
| Prevenile (ISP)*** | (US) | Live Chimera based on the Yellow fever 17D vaccine strain | One single shot Annual vaccination | Side effects | Immunity: 4 weeks after the first injection Efficacy | Safety |

*FDAH-Pfizer: -West Nile Innovator and West Nile Innovator combo are registered in the US
-a West Nile Innovator DNA vaccine is also registered in US but is not commercialized

**Merial: -Recombitek WNV and Recombitek WNV + EWT are registered in the US

*** ISP: -Prestige V + WNV is registered in the US
-Prevenile is registered in the US but withdraw from the market

****BI: -Vetera WNV, VEWT + WNV, EIV + EWT + WNV are registered in the US

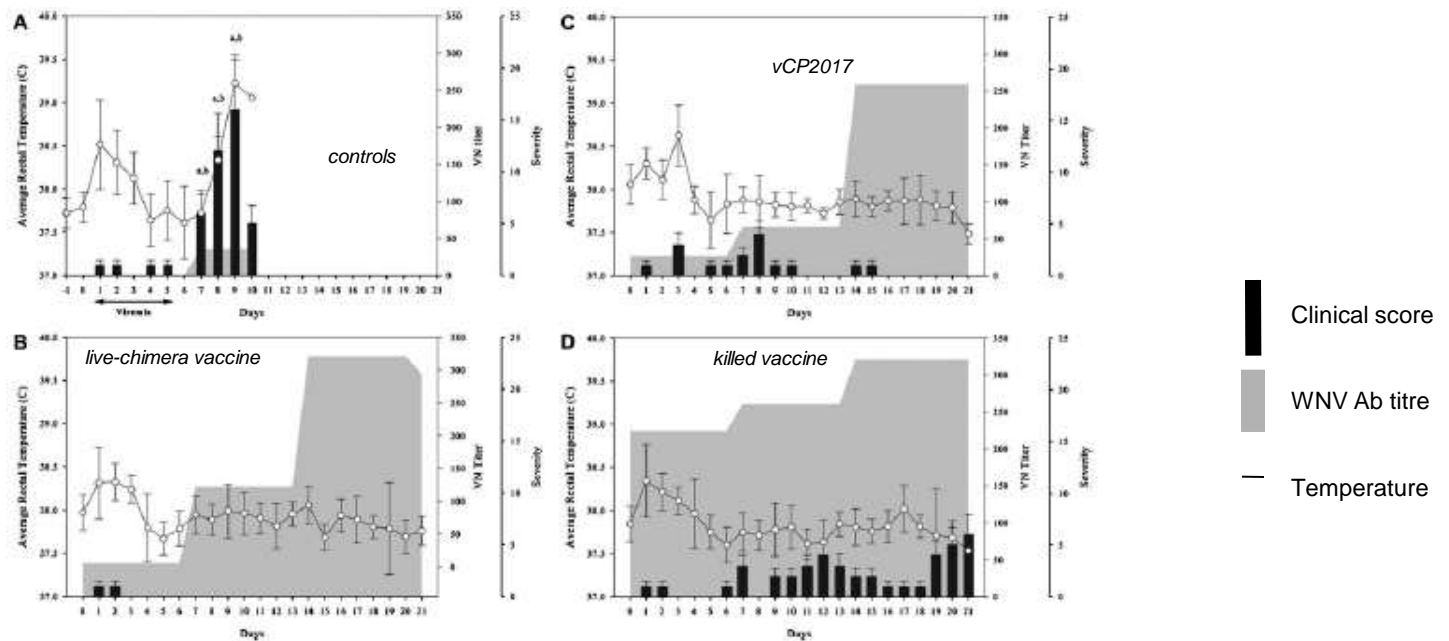
West Nile vaccines: comparative efficacies

Comparative Efficacies of Three Commercially Available Vaccines against West Nile Virus (WNV) in a Short-Duration Challenge Trial Involving an Equine WNV Encephalitis Model^V

K. K. Seino,* M. T. Long, E. P. J. Gibbs, R. A. Bowen,† S. E. Beachboard, P. P. Humphrey, M. A. Dixon, and M. A. Bourgeois

College of Veterinary Medicine, University of Florida, 2015 SW 16th Avenue, Gainesville, Florida 32610

Received 19 June 2007/Accepted 29 July 2007



West Nile vaccines: comparative efficacies

TABLE 1. Summarization of case criteria for vaccinated and control horses after intrathecal WNV challenge

| Vaccine group | No. of horses with criterion ^a | | | | |
|---------------|---|--------------------|---------------------|-----------------|---------------------|
| | Clinical signs | Fever ^e | Death/ ^f | Virus isolation | Histopathic lesions |
| WN-FV | 0/6 | 0/6 | 0/6 | 0/6 | 1/6 ^h |
| CP-WN | 1/5 ^b | 1/5 | 0/5 | 0/5 | 1/5 ^k |
| K-WN | 4/6 ^c | 1/6 | 0/6 | 0/6 | 3/6 ^k |
| Controls | 6/6 ^d | 3/6 | 6/6 | 6/6 | 6/6 ^e |

^a Results are shown as number of horses with criterion/total number of horses.

^b Mild signs in several neurological categories (mentation, paresis, fasciculations, and ataxia) were noted for 1 day.

^c Mild to moderate signs in at least one of the following categories were noted for 1 to 2 days: mentation, paresis, fasciculations, and ataxia.

^d Moderate or severe signs in at least one of the following categories were noted for at least 2 days: mentation, paresis, fasciculations, and ataxia.

^e Fever was indicated by a body temperature of $\geq 39.2^{\circ}\text{C}$ (102.5°F).

^f Death due to development of WNV disease severe enough to require euthanasia for humane reasons.

^h Encephalitic horses in the control group had moderate or severe encephalitis on histopathology.

^k Mild inflammatory histopathologic changes were seen in neural tissues of vaccinated horses.

There is no specific cure for WNV.

Protection against WNV of horses living in or travelling to at risk areas can only be achieved through proper vaccination.

Considering the current epidemiological context in Europe, a good WNV vaccine is one that:

- protects horses against viraemia and clinical disease
- protects against the 2 lineages of the virus
- provides rapid and long lasting immunity
- can be administered to young foals
- does not interfere with epidemiological surveillance testing

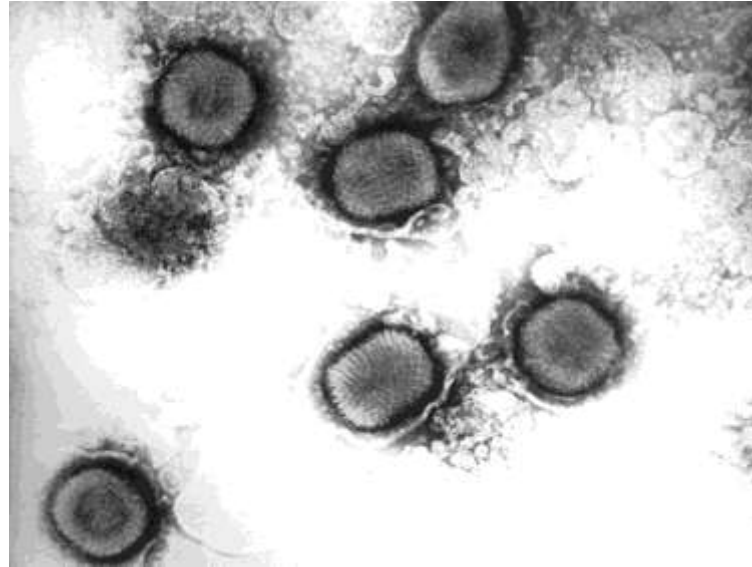
Proteq West Nile – Product Profile

- Tradename : Proteq West Nile
- Canarypox-vectored recombinant vaccine adjuvanted with Carbopol, expressing the prM/E gene from the equine West Nile virus strain
- Liquid vaccine, « ready-to-use », 1ml/dose
- IM route, preferably in the neck region
- Primary vaccination in 2 injections 4-6 wks apart and annual booster with 1 injection
- Targeted Indications and Administration :
 - Active immunisation of horses from 5 months of age against West Nile disease by reducing the number of viraemic horses. If clinical signs are present, their duration and severity are reduced.
 - Onset of immunity: 4 weeks after the first dose of the primary vaccination course. In order to achieve full protection, the full vaccination course of two doses must be given.
 - Duration of immunity: 1 year after a full primary vaccination course of two injections.
- Pack size : 1, 2, 5, 10 doses pack (Marketed packs TBD)
- Targeted shelflife : at least 21 months



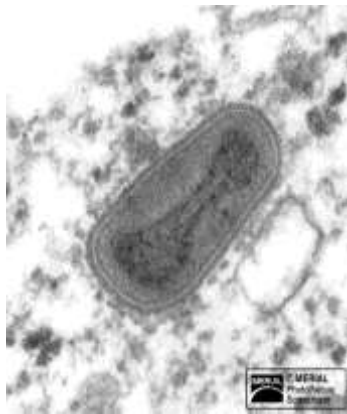
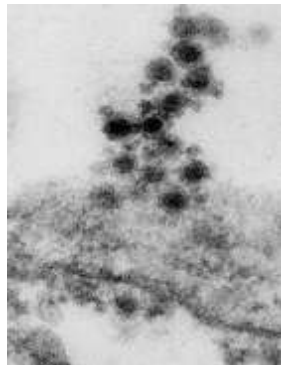
The vector canarypox virus: vCP

- . Double strand DNA genome
- . Large capacity for insertion of foreign genes
- . Cytoplasmic replication in permissive cells

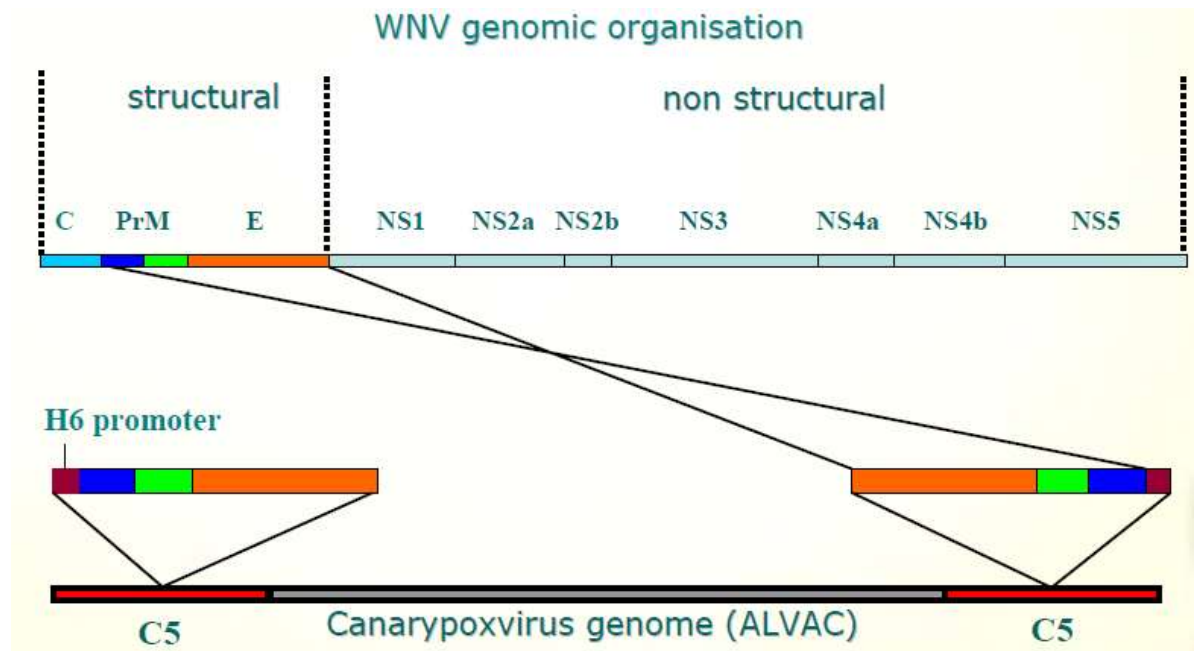


- .Thermostable
- .Genetically stable
- .Replicates only in some cells of avian origin
- .Does not replicate in many bird species
- .Non replicative in mammals in vitro and in vivo

Canarypox West Nile virus construct vCP2017



Donor virus of PrM and E genes: NY99 WNV



Vector virus: Canarypox ALVAC

- **Efficacy**

- Can work with and without adjuvant
- **Provides early onset of immunity**
- Efficacy in presence of Maternally Derived Antibody (MDA)
- **Can be used in a DIVA program**
- Long lasting protection...
- Stimulates B and T cell immunity

- **Safety**

- No shedding
- No risk of reversion to virulence

} **Safe for environment**

- **Convenience**

- May be combined with/and boost other antigens
- Stable (liquid or freeze-dried)
- **No blocking anti-vector immunity**

PROTEQ WEST NILE CLINICAL STUDIES

To demonstrate in laboratory conditions the efficacy of the vaccine on horse, two different models were successfully developed based on two routes of virus administration:

-via WNV-infected mosquitoes (natural model, mosquito).

The natural model (with WNV-infected mosquitoes) was designed to mimic the natural conditions of infection. Similarly to field exposure, it induces viraemia (in 81% of challenged horses), and rarely clinical signs.



-via the intrathecal administration of WNV (experimental clinical model, IT).

This model induces clinical signs such as abnormal mentation, gait deficit, muscle fasciculation, seizure (in 69% of challenged horses), but does not represent the pathogenesis of the disease. It represents a worst case scenario injecting the virus directly into the cerebrospinal fluid of the animals (cisternal space at the atlanto-occipital joint).





Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Protection provided by a recombinant ALVAC[®]-WNV vaccine expressing the prM/E genes of a lineage 1 strain of WNV against a virulent challenge with a lineage 2 strain

J.M. Minke^{a,*}, L. Siger^b, L. Cupillard^a, B. Powers^c, T. Bakonyi^d, S. Boyum^b, N. Nowotny^e, R. Bowen^f

^a Merial S.A.S., 254 rue Marcel Mérieux, 69007 Lyon, France

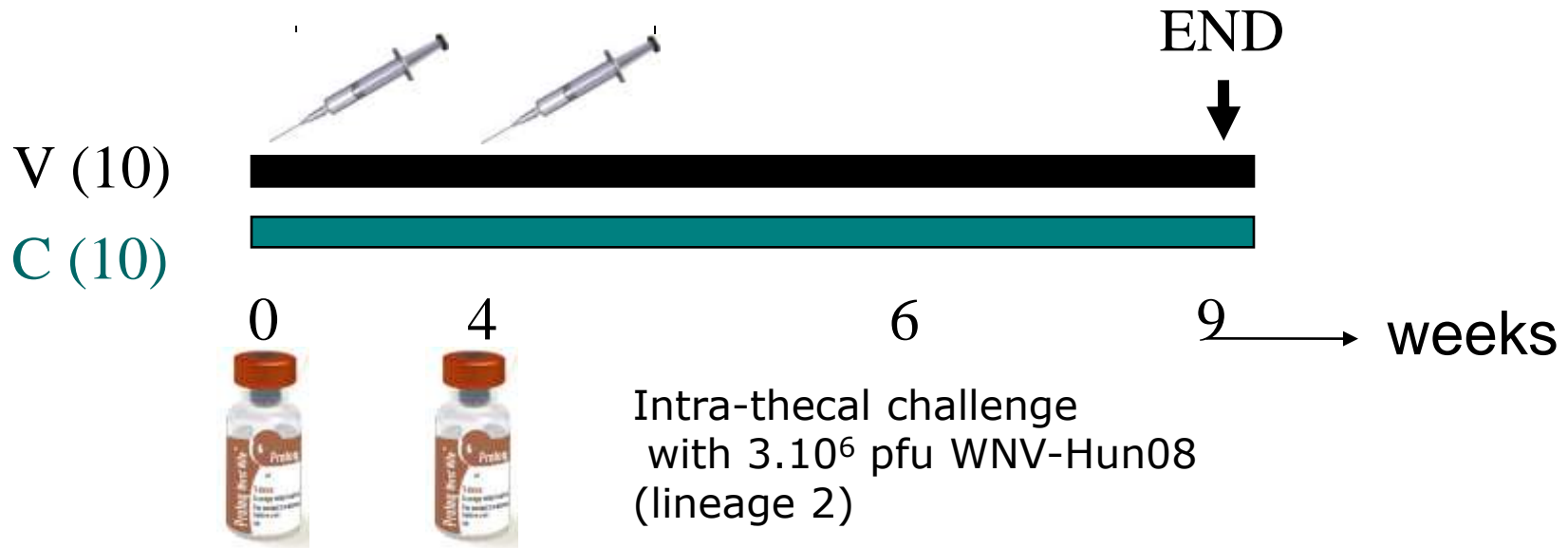
^b Merial Limited, 115 Transtech Drive, Athens, GA 30601, USA

^c 304 Diagnostic Medicine Center, Colorado State University, Fort Collins, CO 80523, United States

^d Department of Microbiology and Infectious Diseases, Faculty of Veterinary Science, Szent István University, Budapest, Hungary

^e Zoonoses and Emerging Infections Group, Clinical Virology, Department of Pathobiology, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria

^f College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80552, USA

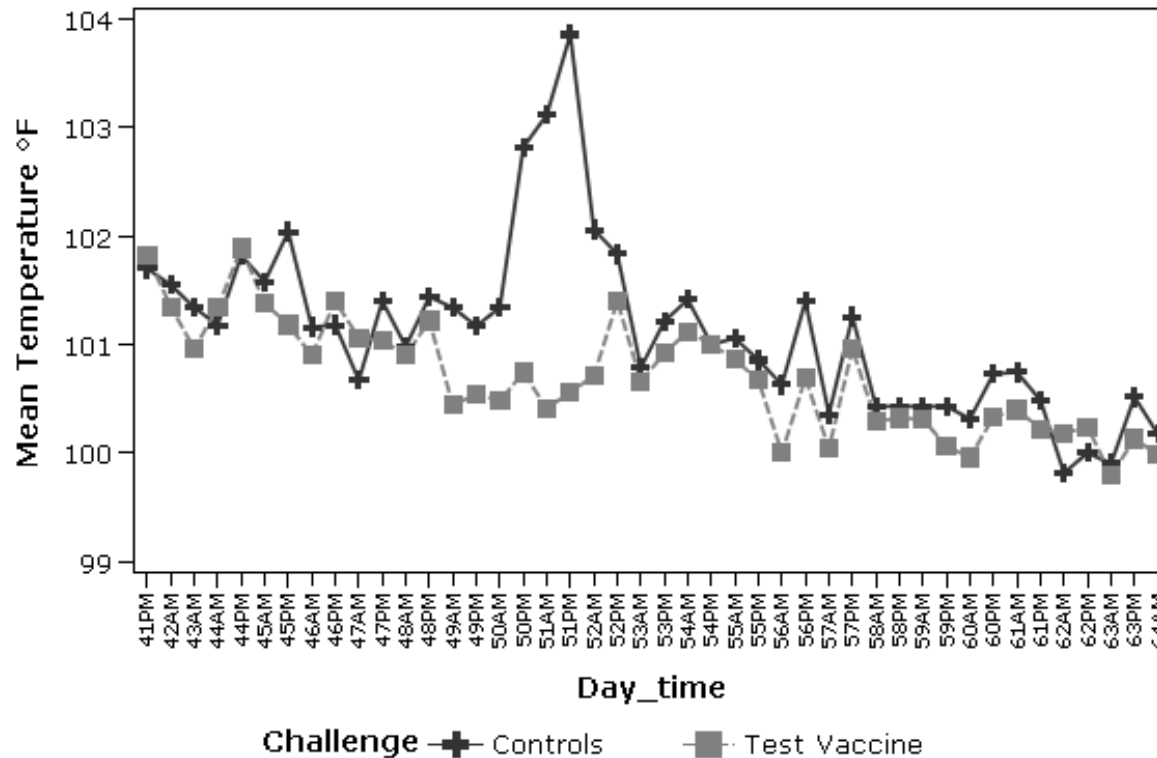


Read out:

- . Incidence of viremia post-challenge
- . Neurological signs
- . Fever

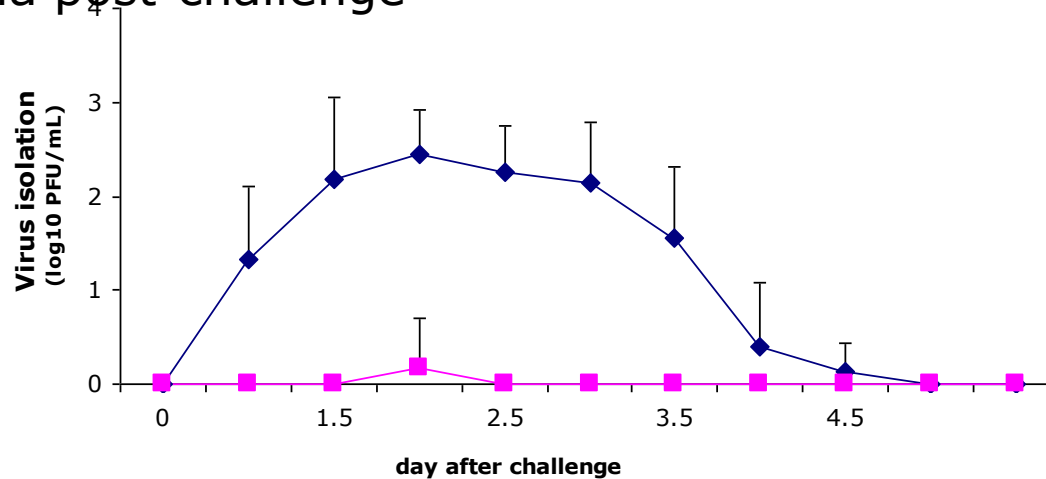


Fever



The temperatures in the control group were significantly higher on Days 49, 50, 51, 52 and 56 (Days 6, 7, 8, 9 and 13 post-challenge)
Hyperthermia $\geq 39.2^{\circ}\text{C}$

Viremia post-challenge



| | Viremia | |
|-------------------------------|----------|----------|
| | Negative | Positive |
| Group_name | | |
| Controls | 0 | 10 |
| Test Vaccine | 9 | 1 |
| Fisher's Exact Test: P=0.0001 | | |

| Prevented Fraction | 95% Confidence Interval |
|--------------------|-------------------------|
| 0.90 | 0.56 - 0.98 |

Clinical signs

| | | Absent | Present | P-value |
|---------------|---|--------|---------|---------------|
| Mortality | C | 7 | 3 | 0.21 |
| | V | 10 | 0 | |
| Mentation | C | 3 | 7 | 0.0031 |
| | V | 10 | 0 | |
| Gait deficits | C | 4 | 6 | 0.01 |
| | V | 10 | 0 | |
| Fasciculation | C | 2 | 8 | 0.0007 |
| | V | 10 | 0 | |
| Recumbency | C | 10 | 0 | N/A |
| | V | 10 | 0 | |
| Lip twitching | C | 6 | 4 | 0.09 |
| | V | 10 | 0 | |
| Head shaking | C | 6 | 4 | 0.09 |
| | V | 10 | 0 | |
| Anorexia | C | 4 | 6 | 0.01 |
| | V | 10 | 0 | |

| Group_name | Disease | | |
|-------------------------------|---------|---|-------|
| Frequency | - | + | Total |
| Controls | 1 | 9 | 10 |
| Test Vaccine | 10 | 0 | 10 |
| Fisher's exact test: P=0.0001 | | | |

| Prevented Fraction | 95% Confidence Interval |
|--------------------|-------------------------|
| 1.00 | 0.68 – 1.00 |

9 out 10 control horses developed WNV disease, while none of the vaccinated horses became sick. The incidence of WNV disease was statistically significantly lower in the vaccinated horses than in the control horses ($P<0.0001$).

OOI: results of experimental clinical model

| Group | Clinical signs | Fever | Viremia |
|--------------------------|----------------|--------------|---------------|
| V | 0/10 | 1*/10 | 1**/10 |
| C | 9***/10 | 9/10 | 10/10 |
| Stat. significant | Yes | Yes | Yes |

*only at two time points

**only at a single timepoint at low titer

***3/10 controls were euthanized

Using a severe challenge model, ALVAC® WNV was shown to significantly prevent the incidence of clinical disease, and viremia against a contemporary neurovirulent lineage 2 WNV isolate, currently circulating in Europe, after a primary course of two doses.

- Thanks to its outstanding vector technology, we believe that Proteq West Nile is the vaccine of choice for protecting horses against the growing threat of WNV in Europe:
 - Protection demonstrated by challenge against both viraemia and clinical signs
 - Protection demonstrated against lineage 1 and lineage 2 of the virus responsible for recent outbreaks of WNV neuroinvasive disease in horses in Europe.
 - Onset of immunity demonstrated 4 weeks after a single dose
 - Safety confirmed in foals as young as 2 months of age
 - Can be used in pregnant and lactating mares
 - Induction of both WNV neutralizing antibodies and Cell-mediated immunity
 - True DIVA potential

The vaccine provides veterinarians with an important tool in controlling WNV infection during a natural outbreak. It is also based on the demonstration of the efficacy and the safety of the vaccine for years in the USA in strong endemic conditions.



Acknowledgments:

- Norbert Nowotny, Vetmeduni Vienna, Austria
- Richard Bowen, Colorado State University, Fort Collins, USA
- MERIAL R&D, Lyon, Gerland, France: L. Gahinet, V. Woerly, M. Henaff, L. Siger, D. Calmels, D. Corneille, J. Minke

The canarypox platform is now used for a variety of Veterinary Vaccines

