

"Le virus de la Fièvre de la Vallée du Rift : revue à partir de données nouvelles »

Michèle BOULOY

Unité de Génétique Moléculaire des Bunyavirus



The Bunyaviridae family

There are more than 400 known members grouped into 5 genera

- **Orthobunyavirus:** *Bunyamwera virus*
- **Phlebovirus:** *Rift Valley fever virus*
- **Nairovirus:** *Nairobi sheep disease virus*
- **Tospovirus:** *Tomato spotted wilt virus*

Arboviruses



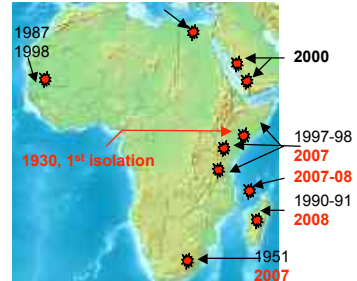
- **Hantavirus:** *Hantaan virus*

Rodent borne viruses

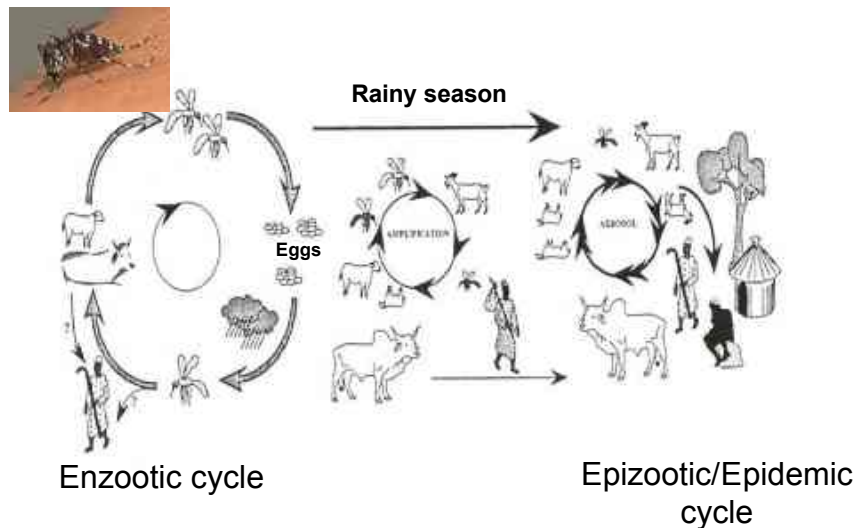


Rift Valley fever

- Zoonosis affecting humans and ruminants in Africa and Yemen and Saudi Arabia since 2000
- Virus transmitted by many species of mosquitoes
- Hemorrhagic fever in humans and hepatitis, abortion and death in ruminants
- No safe vaccine for protection nor antiviral agents for therapy
- Potential bioterrorism agent



Transmission of RVF



(Zeller *et al.*, 1997)

LA FIÈVRE DE LA VALLÉE DU RIFT **ÑAWU WALEE RIFT**

C'EST UNE ZOOSE MATEURE
ELLE AFFECTE AUSSI BIEN LES ANIMAUX QUE LES HOMMES.

RAWU KEENGU ADDUDE WOPPERE NDER ZIBINGOL, KAM E WARDE JAWDI TOKOSIRI NDI
ÑAWU NSIL ÑA ARA E JAWDI, ÑA ARA MAI E NEDDO.

• MANIFESTATIONS • NO DU SIFORTEE ?

• CHEZ LES ANIMAUX • TO JAWDI TOO

- BEUCOUP D'AVORTEMENTS - ÑA NEEWI ADDUDE WOPPERE (WERLERE)
- FORTE MORTALITÉ DES AGNEAUX, CHEVREAUX ET VEAUX.
- ÑA NEEWI MORDRE JAWDI WALLA MAMMARI TOKOSIRI NDI

• CHEZ L'HOMME • TO NEDDO TOO

- FORTE FIÈVRE, RESSEMBLANT AU PALUDISME OU À LA FIÈVRE JAUNE
- 40°
- GANNDU WILLA YILLEE ÑA ARA WAM NO GARANDU JONTINGOJE

From Y. Thiongane ISRA, Senegal

Rift Valley fever virus
(Bunyaviridae, Phlebovirus)

Freiberg et al 2008

genome

- L segment: 6404 nt, L protein
- M segment: 3885 nt, Precursor to Gn, Gc, 14 K, & 78K
- S segment: 1690 nt, NSs protein, N protein

Tree based on NSs sequence

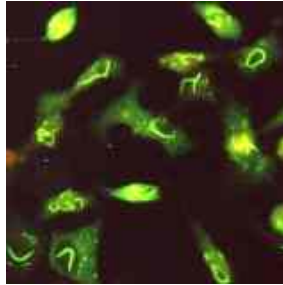
- East-Central Africa:** Ar SEN 93, H1 MAU 87, Ar RCA 69, Ar UGA 55, SNS, An MAD 91, H KEN 98, Ar SAU 01, H CHA01
- West Africa:** H2 MAU 87, H3 MAU 87, H4 MAU 87, An SENK 93, Ar BUF 84, Ar GUI 83
- Egypt:** H EGY 77, H/B EGY 93, MP 12, Ar SEN 83, Ar MAD 79

Diagram of the virus structure: A spherical virus with a penton and hexon surface. The genome is segmented into L, M, and S segments. The surface proteins are N, L, Gn, and Gc.

Electron micrographs: L, M, and S segments.

NSs forms filamentous structures in the nucleus in spite of the fact that all the steps of the viral cycle occur in the cytoplasm

Immunofluorescence with anti-NSs antibodies

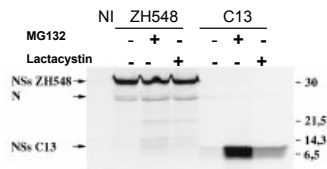
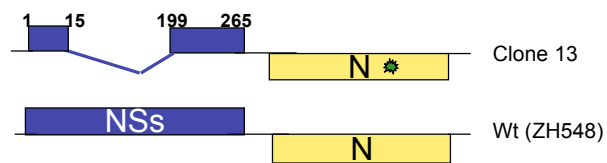


Section of a RVFV infected cell Electron microscopy



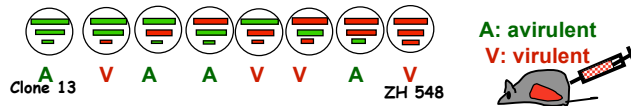
NSs is an accessory protein

Clone 13 has an in frame internal deletion of 70% of NSs ORF. The truncated protein remains in the cytoplasm and is degraded by the proteasome



NSs is the major virulence factor

- Clone 13 is avirulent for mice: its S segment carries a major determinant for attenuation

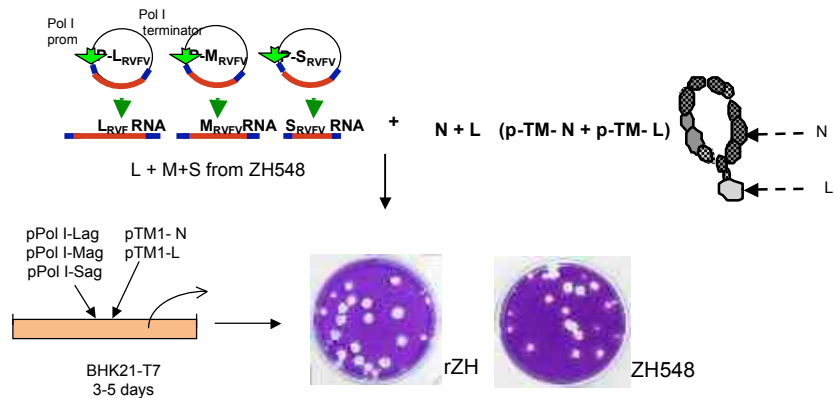


- Interferon α/β plays a major role for attenuation
 - Clone 13 caused a rapid death in type I IFN receptor deficient mice
 - Clone 13 induced a high titer of IFN in the serum of infected mice whereas the virulent ZH548 did not

→ Role of type I interferon for attenuation

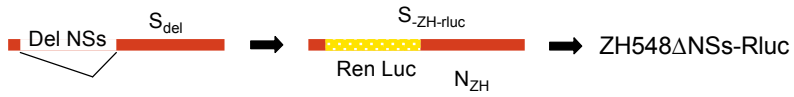
Reverse genetics

Reconstitution of viral-like RNPs active for transcription and replication



Bioluminescence imaging in living mice

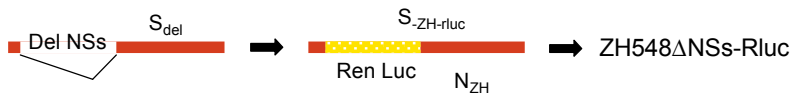
L + M from ZH548 and S from rLuc-ZH



ZH548 Δ NSs-Rluc is avirulent in normal mice...

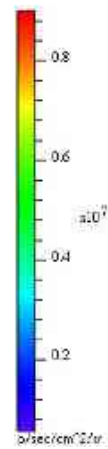
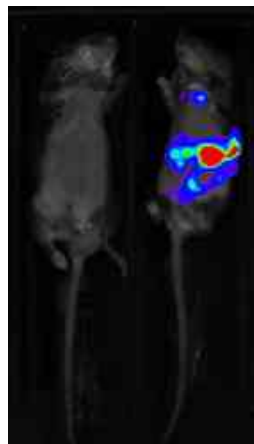
Bioluminescence imaging in living mice

L + M from ZH548 and S from rLuc-ZH



ZH548 Δ NSs-Rluc is avirulent in normal mice... but pathogenic in *ifnar*^{-/-} mice

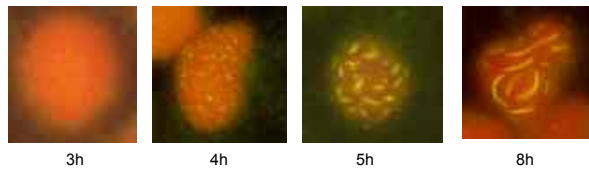
16h p.i.



Collaboration JJ Panthier and C. Gomet

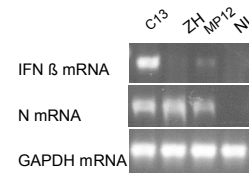
NSs is involved in

- ✓ filament formation



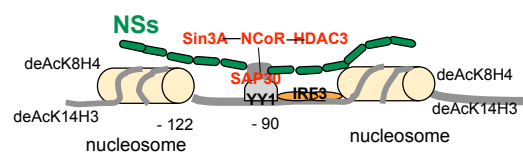
NSs is involved in

- ✓ filament formation
- ✓ inhibiting IFN- β production

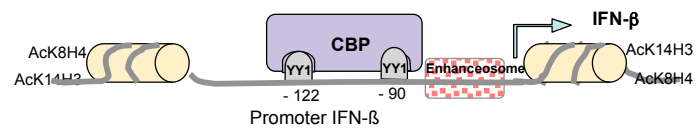


NSs inhibits IFN transcription by interacting with SAP30 of the Sin3A repression complex

RVFV ZH infected cells :

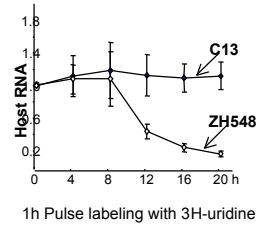


RVFV C13 infected cells :

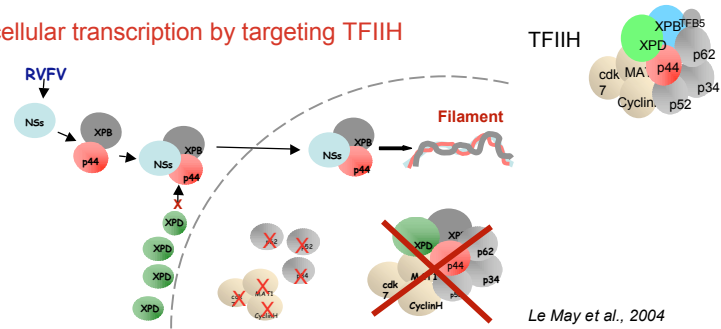


NSs is involved in

- ✓ filament formation
- ✓ inhibiting IFN- β production
- ✓ Inhibiting cellular transcription

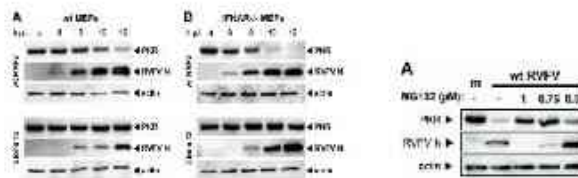


NSs inhibits cellular transcription by targeting TFIIH



NSs is involved in

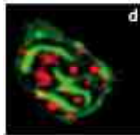
- ✓ filament formation
- ✓ inhibiting IFN- β production
- ✓ Inhibiting cellular transcription
- ✓ Degrading PKR (*Habjan et al 2009, Ikegami et al 2009*)



Habjan et al 2009

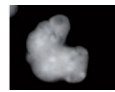
NSs is involved in

- ✓ filament formation
- ✓ inhibiting IFN- β production
- ✓ Inhibiting cellular transcription
- ✓ Degrading PKR (*Habjan et al 2009, Ikegami et al 2009*)
- ✓ Interacting with pericentromeric gamma satellite sequence and inducing chromosomes segregation defects

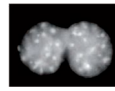


Immuno-FISH
NSs=green
Gamma satellite seq=red

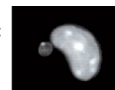
Lobulated nuclei:



Intranuclear bridge:



Micronuclei:



Rationale for the design of attenuated vaccines

The lack of NSs benefits to the host, as it allows an efficient innate response

Licensed veterinary vaccine: Smithburn Neurotropic Strain (SNS)

Obtained by intracerebral passages of the virulent strain Entebbe in suckling mice (*Smithburn, 1949*). **Is immunogenic but has secondary effects (neurotropism, abortigenic, teratogenic 15%)**



Candidate vaccine: MP12

Derived from a virulent strain isolated in Egypt in 1977 (ZH548) and attenuated by serial alternating passages in the presence or the absence of 5-fluorouracil (*Caplen, Peters & Bishop, 1985*). **Has similar secondary effects (Teratogenic 14%) (Hunter, Erasmus & Vorster, 2002)**

Naturally attenuated strain: C13

A plaque Isolated from a benign human case in Centre Afrique Republic (*Muller et al., 1995*) **appears as a good candidate as it NSs is defective**

Clone 13 Vaccination trials

Carried out by OBP in BSL3 Stables

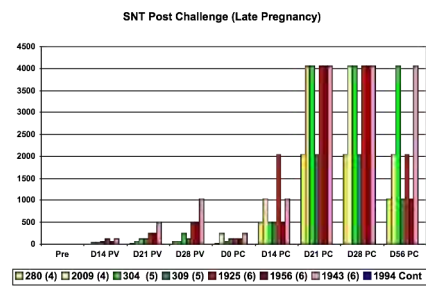
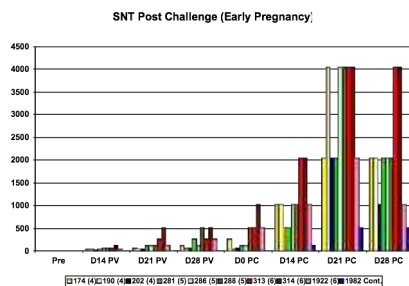
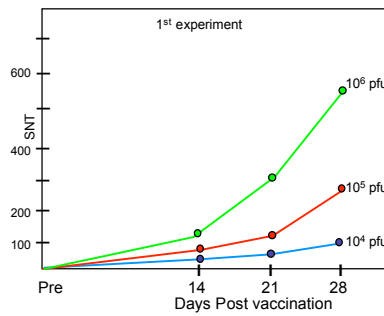


Vaccination of sheep

- analysis of dose (10^4 , 10^5 and 10^6 pfu) effect and immunogenicity

Vaccination of ewes with 10^4 , 10^5 and 10^6 pfu to assess

- immunogenicity
- effect on pregnancy, teratogenicity and lambing tested at two stages (30 and 100 days). Synchronization of oestrus and artificial insemination
- protective effect against a virulent challenge (at 30 d or 90 d. post vaccination)



Summary

3 successful trials in 34 pregnant ewes: vaccination at different stages of pregnancy;

- No abortion in pregnant ewes vaccinated at different stages (30 to 100 days)
- Protection against abortion after virulent challenge in vaccinated while all control aborted
- No evidence of shedding & horizontal transmission of the virus as no unchallenged control seroconverted while being housed with vaccinated ewes

•Efficacy

- Protective dose determined
- No viraemia detected post-challenge
- Long term neutralizing antibodies
- Good maternal antibody levels in offsprings

•Finalizing registration in South Africa

•Ready for field trials in endemic areas !!!

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Vaccination trials

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